University of Alberta

Examination of Physiological and Morphological Parameters of a Population of Lodgepole Pine (*Pinus contorta* Dougl. spp. *latifolia*) Seedling Roots in Relation to First Year Seedling Growth

by

Veronika Lukic

©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Plant Science

Department of Agricultural, Food and Nutritional Science

Edmonton, Alberta

Fall, 1997

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-22629-8

Canada

University of Alberta

Library Release Form

Name of Author:	**Veronika Lukic**
Title of Thesis:	**Examination of Physiological and Morphological Parameters of a Population of Lodgepole Pine (*Pinus contorta* Dougl. spp. *latifolia*) Seedling Roots in Relation to First Year Seedling Growth**
Degree:	**Master of Science**
Year of degree:	**1997**

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly, or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Veronika Lukic

8347-76 Avenue
Edmonton, AB
Canada, T6C 0H9

Date: October 3, 1997

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Examination of Physiological and Morphological Parameters of a Population of Lodgepole Pine (*Pinus contorta* Dougl. spp. *latifolia*)) Seedling Roots in Relation to First Year Seedling Growth** submitted by **Veronika Lukic** in partial fulfillment of the requirements for the degree of **Master of Science** in Plant Science.

Dr. A. M. Johnson-Flanagan

Dr. M. Jones

Dr. J. Zwiazek

Dr. F. Yeh

Date: September 1997

For my mother Ana, for her encouragement

ABSTRACT

The relationships between morphological and physiological parameters in lodgepole pine (*Pinus contorta* Dougl. spp. *latifolia*) seedlings in relation to outplanting success and subsequent field performance was studied by assessing root morphology, root growth capacity (RGC), viability (TTC reduction), reducing, total soluble and total non-structural carbohydrates, starch, and drought tolerance in seedlings before and after cold storage. The effect of slow and fast cooling as seedlings enter into cold storage was also examined. Seedling survival and subsequent field performance was best predicted by TTC reduction prior to cold storage. RGC was also a good predictor; however, the test takes far longer, requires more space and must be done after cold storage. Drought stress tolerance, measured as RGC or needle injury, correlated with outplanting survival and field performance. The relationship between drought stress tolerance, measured as electrolyte leakage, and carbohydrates was also examined. Both total soluble and total non-structural carbohydrates were correlated with root injury.

ACKNOWLEDGMENTS

There are many individuals whose efforts have made a significant contribution to my research. First, I would like to thank my supervisor, Dr. Anne Johnson-Flanagan, for her guidance and financial support during the course of my program, and particularly for her time and energy directed towards reviewing the thesis. I am grateful to my co-supervisor, Dr. Melanie Jones, for her encouragement and thoughtful guidance of my research during my stay at Okanagan University College, Kelowna, B.C. My committee members Dr. Janusz Zwiazek and Dr. Francis Yeh have been an invaluable resource, and I appreciate their insight. I would like to thank Rob Scagel from Pacific Phytometric Consultants, Surrey, B.C., for his contribution in field observations and for his support and advice throughout the project. For their very helpful support, I would like to extend my sincere appreciation to Trevor Carnelley and Michele Konschuh. I would like especially to thank Dr. Dan Durall, Dr. Debbie Classen, Shannon Hagerman and Michelle Harniman for their help and support during my stay at Okanagan University College, Kelowna, B. C., and Tania Perzoff from University of British Columbia, Vancouver, B.C. for her great assistance in field work. Finally, I would like to thank my family and my husband for their understanding and encouragement.

TABLE OF CONTENTS

Chapter	Page Number
List of Tables	
List of Figures	
1. Introduction	
1.1 Problems in Reforestation	1
1.2 Research Objectives	2
1.3 Literature Cited	4
2. Literature Review	
2.1 Root Morphology	5
2.2 Root Growth Capacity (RGC)	6
2.3 Root Viability	7
2.4 Needle Morphology	8
2.5 Chlorophylls	8
2.6 Carbohydrates	9
2.6.1 Root Carbohydrate	10
2.6.2 Needle Carbohydrate	10
2.7 Growth Cycle	11
2.8 Chilling Requirement	11
2.9 Lifting and Cold Storage	12
2.10 Drought and Desiccation Stress Tolerance	13
2.11 Electrolyte Leakage	15
2.12 Literature Cited	17
3. Root Growth and Morphology, Carbohydrate Reserves and Field Performance of Lodgepole Pine Seedlings	
3.1 Introduction	26
3.2 Materials and Methods	27
3.2.1 Plant Material	27
3.2.2 Pre-storage Sampling	27
3.2.3 Cold Storage, Thawing Conditions and Post-Cold Storage Sampling	28
3.2.4 Viability Testing (TTC)	28
3.2.5 RGC Testing	29
3.2.6 Extraction of Carbohydrates	29
3.2.6.1 Reducing Carbohydrate Content	29
3.2.6.2 Soluble Carbohydrate Content	30
3.2.6.3 Starch Content	30

3.2.6.4 Quantification 31
3.2.7 Outplanting 32
3.2.8 Statistical Analysis 33
3.3 Results 34
3.3.1 Root Morphology 34
3.3.2 Root Carbohydrate 38
3.3.3 Survival and Growth Parameters After the First Growing Season 44
3.4 Discussion 48
3.5 Literature Cited 56

4. Effects of Slow and Fast Cooling on Root Growth and Morphology, and Root and Needle Carbohydrate Reserves in Lodgepole Pine Seedlings

4.1 Introduction 59
4.2 Material and Methods 60
4.2.1 Plant Material 60
4.2.2 Pre-storage Sampling 60
4.2.3 Post-storage Sampling 61
4.2.4 Sampling for Root Carbohydrate and Starch 63
4.2.5 Needle Reducing Carbohydrate and Total Soluble Carbohydrate Content 63
4.2.6 Needle Starch Content 63
4.2.7 Total Chlorophyll, and Chlorophyll a and b Content 63
4.2.8 Outplanting 64
4.2.9 Statistical Analysis 64
4.3 Results 65
4.4 Discussion 73
4.5 Literature Cited 78

5. *In vivo* Study of Lodgepole Pine Seedling Drought Tolerance

5.1 Introduction 81
5.2 Material and Methods 82
5.2.1 Plant Material 82
5.2.2 Development of Drought Experimental Design 83
5.2.3 Pre-storage Experiment 83
5.2.4 Post-storage Experiment 83
5.2.5 Moisture Content of Substrate (MC) 83
5.2.6 Moisture Content of Needles (MC) 84
5.2.7 Electrolyte Leakage of Needles 84
5.2.8 Electrolyte Leakage of Roots 84
5.2.9 Root Growth Capacity (RGC) 85
5.2.10 Survival Rate 85

5.2.11 Statistical Analysis 85
5.3 Results 86
5.3.1 Preliminary Study 86
5.3.2 1995 Study 86
5.3.3 1996 Study 92
5.3.4 Statistical Analysis of 1995 and 1996 Results 96
5.4 Discussion 99
5.5 Literature Cited 103
6. Summary and Conclusions 105
7. Appendix 107

LIST OF TABLES

Table **Page Number**

Table 3.1: Lifting and outplanting dates..... 32

Table 3.2: Correlation coefficients for reducing carbohydrate, total soluble carbohydrate, starch, total non-structural carbohydrate and RGC prior to and after cold storage..... 45

Table 3.3: Correlation coefficients for reducing carbohydrate, total soluble carbohydrate, starch, total non-structural carbohydrate and RGC prior to and after cold storage in 1995 and 1996..... 46

Table 3.4: Field results of 1995 crop outplanted in spring of 1996..... 47

Table 3.5: Correlation coefficients for root morphological parameters with field performance..... 49

Table 3.6: Linear regression equations of viability (before and after regrowth and before and after storage) and survival, collar diameter and height increment..... 50

Table 3.7: Correlation coefficients between carbohydrates and field performance..... 51

Table 4.1: Lifting and outplanting dates in 1996..... 62

Table 4.2: Fluctuation in total soluble carbohydrate during cold storage..... 69

Table 5.1: Lifting dates..... 82

Table 5.2: Parameters tested in order to determine the drought stress regime..... 87

Table 5.3: Survival rate of seedlings (following storage) after a 10 day drought stress and regrowth for 7 days under RGC conditions..... 91

Table 5.4: Correlation coefficients for RGC and root injury with root reducing carbohydrate, total soluble carbohydrate, starch and total non-structural carbohydrate following cold storage and drought stress in 1995..... 91

Table 5.5: Correlation coefficients between drought stress parameters measured after cold storage and field performance for lodgepole pine seedlings lifted in 1995..... 93

Table 5.6: Survival rate of seedlings (prior to and after storage) after a 10 day drought stress and regrowth for seven days under optimal conditions..... 98

Table 5.7: Correlation coefficients for RGC and root injury with reducing carbohydrate, total soluble carbohydrate, starch and total non-structural carbohydrate following cold storage and drought stress in 1996..... 98

Table 5.8: Linear regression equations of RGC and root injury in 1995 and 1996 following drought stress after cold storage..... 99

Table 5.9: Linear regression equations of root injury with total soluble and total non-structural carbohydrates in 1995 and 1996 following drought stress after cold storage..... 100

LIST OF FIGURES

Figure **Page Number**

Figure 3.1: Root morphology data for lodgepole pine seedlings prior to cold storage. 35

Figure 3.2: Root morphology data for lodgepole pine seedlings after cold storage..... 36

Figure 3.3: Viability and RGC for lodgepole pine seedlings prior to and after cold storage..... 37

Figure 3.4: Content of reducing carbohydrates in roots of lodgepole pine seedlings.. 39

Figure 3.5: Content of total soluble carbohydrate in roots of lodgepole pine seedlings. 40

Figure 3.6: Starch content in roots of lodgepole pine seedlings..... 42

Figure 3.7: Content of total non-structural carbohydrate in roots of lodgepole pine seedlings..... 43

Figure 4.1: Slow and fast cooling rates..... 61

Figure 4.2: Root morphology data for lodgepole pine seedlings prior to and after cold storage..... 66

Figure 4.3: Root morphology data and RGC for lodgepole pine seedlings prior to and after cold storage..... 67

Figure 4.4: Root morphology following RGC for lodgepole pine seedlings prior to and after cold storage..... 68

Figure 4.5: Carbohydrate content in lodgepole pine seedling roots..... 70

Figure 4.6: Carbohydrate content in needles of lodgepole pine seedlings..... 72

Figure 4.7: Chlorophyll a and b content in needles of lodgepole pine seedlings..... 74

Figure 4.8: Total chlorophyll and chlorophyll a/b ratio in needles of lodgepole pine seedlings..... 75

Figure 5.1: Response of lodgepole pine seedling needles to drought stress following cold storage..... 88

Figure 5.2: Response of lodgepole pine seedling roots to drought stress following cold storage..... 90

Figure 5.3: Response of lodgepole pine seedling needles to drought stress..... 94

Figure 5.4: Response of lodgepole pine seedling roots to drought stress..... 95

Figure 5.5: Response of lodgepole pine seedling roots to drought stress..... 97

CHAPTER I

1. INTRODUCTION

1.1 Problems in Reforestation

Regeneration or creation of new coniferous forests usually requires nursery-produced seedlings. These seedlings are subjected to different stresses from the time of nursery lifting, to planting in the field, and thereafter. Once in the field, growth and survival of young seedlings depends on the interactions between seedling performance potential and site constraints.

Seedlings used in reforestation are grown in nurseries as either bareroot or containerized crops. Often they are sown in early spring and are lifted to cold storage (-2°C) in the fall prior to outplanting the following summer. Cold storage enables the gap between fall or winter lifting and spring planting to be bridged. The timing of cold storage is critical to the success of the crop, as seedlings that are lifted too early or too late have been shown to have reduced viability and perform poorly following outplanting (Omi et al., 1994). Thus, it is essential that rapid, inexpensive and accurate methods to assess the best time for lifting be developed.

Transplanting stress is a normal consequence of lifting, handling, storing, shipping and planting seedlings into a field environment, even under ideal planting conditions. To survive transplanting, the seedling must recover from the injuries and stresses that result from lifting and handling, resist desiccation stresses and grow new roots to avoid drought stress, continue maintenance and growth respiration, and adapt to a more hostile environment. The seedlings must do all of this before carbohydrate reserves are exhausted. A successful seedling is able to restore normal plant water relations, produce sufficient new photosynthate to support root and shoot growth, and restore carbohydrate reserves. This is best accomplished by seedlings with high drought tolerance (Hallgren, 1991).

Lodgepole pine (*Pinus contorta* Dougl.) is one of the most widely distributed and diverse native species in western North America. The coastal variety of the species can be

found at elevations ranging from sea level to 600 m. The inland variety grows from 500 to 1,000 m in the north and from 2,000 to 3,500 m in the south (Whitney, 1985). It is an economically important reforestation species, not only in the USA and western Canada, but also in Scandinavia, England and New Zealand (Koch, 1996). This is because lodgepole pine grows quickly from the time of planting, and is capable of producing large trees in a short period of time.

Lodgepole pine is one of the most drought and frost-tolerant, but shade-intolerant native conifers (Eremko, 1990). It can occupy very infertile sites, probably through its extremely low nutrient requirements, or through its ability to extract nutrients unavailable to other species (Lotan and Perry,.1983). However, lodgepole pine is a poor competitor for nutrients especially in competition with grass species. Lodgepole pine seedlings are also susceptible to being trampled and eaten by cattle (Lotan and Perry, 1983).

The average survival rate of lodgepole pine seedlings after outplanting in British Columbia is high (85%) (Eric van Steenis, pers. comm.). Although a 15% mortality rate seems low, when one considers that millions of seedlings are planted each year, any loss is significant. More importantly, the potential losses resulting from poor seedling growth after outplanting are expected to be very high.

1.2 Research Objectives

Prior to implementing reforestation management strategies for improving lodgepole pine seedlings, rapid, direct predictors of survival and growth after outplanting need to be determined. My hypothesis is that carbohydrates will be faster to measure and be a better indicator than is RGC. The first objective was to study the gross morphology of the root system, root growth, TTC reduction, RGC and carbohydrate reserves before outplanting and to compare them to seedling survival and establishment following outplanting.

My second hypothesis is that slow cooling and thawing (common practices in the industry) contribute to reductions in seedling health following outplanting. Seedlings are frozen in boxes of 250 seedlings. This means that only the seedlings on the outside of the box freeze and thaw quickly, while those in the middle of the box freeze and thaw more

slowly. It is not unusual for seedlings to remain in the unfrozen state in cold storage for a period of weeks after lifting (Anne Johnson-Flanagan per. comm.). One would expect the seedlings to remain metabolically active during this time. As such, the question arises "How does the rate of cooling affect carbohydrate status and subsequent seedling health after storage?" As a first step to gaining insight into this, my objective was to examine the effect of slow and fast cooling and thawing on parameters including root morphology, and root and needle storage reserves.

The final hypothesis is that high drought tolerance will correlate with good seedling growth after outplanting and that high total soluble carbohydrate content will correlate with high drought tolerance. The final objectives were, therefore, to develop the methodology for *in vivo* assessment of drought tolerance, to relate stress tolerance to characteristics measured above and to examine the relationship between drought tolerance and seedling productivity in the first season after outplanting.

To date, much of the research on seedling quality has focused on outplanting success in terms of survival and not on seedling (tree) productivity (Deans et al., 1990; Omi et al., 1994; Sharpe and Mason, 1992; van den Driessche, 1991, 1992). The present study is part of a larger study that will assess seedling growth parameters for up to 3 years following outplanting. In this manner we will be able to assess the utility of the various biochemical and morphological parameters measured in relation to outplanting success, seedling establishment and tree growth.

1.3 Literature Cited

Deans, J.D., Lundberg, C., Tabbush, P.M., Cannell, M.G.R., Sheppard, L.J., and Murray, M.B. 1990. The influence of desiccation, rough handling and cold storage on the quality and establishment of Sitka spruce planting stock. Forestry. 63:129-141.

Eremco, D.R. 1990. A review of natural Lodgepole pine regeneration in the Chilcotin. FRDA. Report 126.

Hallgren, S.W., Tauer, C.G., and Lock, J.E. 1991. Fine root carbohydrate dynamics of Loblolly pine seedlings grown under contrasting levels of soil moisture. For. Sci. 37:766-780.

Koch, P. 1996. Lodgepole pine commercial forests: an essay comparing the natural cycle of insect kill and subsequent wildfire management for utilization and wildlife. Gen. Tech. Rep. INT-GRT-342, Ogden. UT:U.S. Department of Agriculture Forest Services, Intermountain Research Station, p.24.

Lotan, J.E., and Perry, D.A. 1983. Ecology and regeneration of Lodgepole pine. U.S. Dep. Agric. For. Serv., Washington, D.C. Agric. Handb. p. 606.

Omi, S.K., Rose, R., and Sabin, T.E. 1994. Fall lifting and long-term freezer storage of ponderosa pine seedlings: effects on starch, root growth, and field performance. Can. J. For. Res. 24:624-637.

Sharpe, A.L., and Mason, W.L. 1992. Some methods of cold storage can seriously affect root growth potential and root moisture content and subsequent forest performance of Sitka spruce and Douglas fir transplants. Forestry, 65:463-476.

van den Driessche, R. 1991. Influence of container nursery regimes on drought resistance of seedlings following planting. I. Survival and growth. Can. J. For. Res. 21:555-565.

van den Driessche, R. 1992. Changes in drought resistance and root growth capacity of container seedlings in response to nursery drought, nitrogen, and potassium treatments. Can. J. For. Res. 22:740-749.

Whitney, S. 1985. Western Forests. Charitcleer Press, Inc., New York. pp. 356-411.

CHAPTER II

2. LITERATURE REVIEW

2.1 Root Morphology

To date, there has been little research on gross morphology of conifer root systems in relation to planting success and superior tree productivity. In studies on white spruce (*Picea glauca*), Johnson-Flanagan and Owens (1985 a,b) found that root quality (proportion of elongating, absorbing and brown roots) may be more important than quantity of white roots (root growth capacity) with regard to planting success. They determined that lateral root elongation early in the spring and under optimum conditions enlarged the root system. Later, the proportion of absorbing roots (hair-covered roots) increased as the root growth capacity decreased. Research by Stone et al. (1962) indicated that high planting success correlated with the spring time decrease in root growth capacity. This implies that root class, and more specifically, the preponderance of absorbing roots may be more important than root biomass in determining seedling survival (Johnson-Flanagan and Owens, 1985b).

Based on external morphology, Johnson-Flanagan and Owens (1985a) described three classes of lateral roots in white spruce. Elongating roots have few root hairs and are characterized by rapid rates of elongation. Absorbing roots are characterized by root hairs in the zone of elongation and differentiation and are usually short. They are responsible for nutrient uptake (Lyr and Hoffman, 1972). As such, the seedling relies upon a large portion of absorbing roots for proper growth. Growth of absorbing roots is determinate - the roots die after one growth cycle (Rose, 1992). Brown roots are enveloped by a necrotic layer and are elongating roots between periods of growth. When elongation ceases, roots become brown as a result of suberization of the endodermis. They may be quiescent or dormant. This does not mean they are metabolically inactive. Johnson-Flanagan and Owens (1986) demonstrated that brown roots of white spruce continued to respire at 47 percent of the rate of elongating roots.

Growth cycles of individual roots of the elongating class are independent. The mechanisms controlling growth cycles are complex and little understood, but evidence

provided by Wilcox (1968), from his studies with red pine (*Pinus resinosa*), strongly suggests that one of the controlling mechanisms involves soil-plant water relations. Under conditions of continuous moisture, elongation occurs for 2 to 3 weeks, slows and then ceases. The cessation of growth leads to browning of the root in an acropetal direction. This is caused by the formation of the secondary endodermis and the metacutization layer, which together, form the "dormancy layer". Formation of the dormancy layer is associated with accumulation of starch in the root tip and pericycle (Johnson-Flanagan and Owens, 1985a; Wilcox, 1954).

Cell divisions precede visible growth (Johnson-Flanagan and Owens, 1985a). The first sign of visible growth is swelling of the root apex. This is concomitant with loss of the starch sheath, indicating that starch to sugar conversions may play a role in cell expansion. Studies on the zone of elongation in dicot roots support this concept. Increased invertase activity accompanies accumulation of reducing sugars in expanding cells (Sutcliffe and Sexton, 1968). Emergence of a white apex through the brown layer indicates the resumption of elongation.

2.2 Root Growth Capacity (RGC)

Root growth capacity (RGC) can be defined as a seedling's ability to initiate root growth under optimal conditions (Stone and Jenkinson, 1971). Variation in RGC occurs seasonally (Stone et al., 1962), in response to cultural treatments such as fertilization (van den Driessche, 1988), from damage caused by frost (Colombo et al., 1984), or from lifting, handling and storage (Tabbush, 1986). Lifting and storage schedules are often based on RGC (Tabbush, 1988).

Laboratory assays for measuring the RGC of forest tree seedlings were first developed in the belief that root extension immediately after planting is a major determinant of establishment success. Another assumption underlying the development of these tests is that root growth under standardized conditions in the laboratory is indicative of root growth under field conditions (Burdett, 1987; Ritchie and Tanaka, 1990). While this test has proven to be a useful predictor for seedling survival in some cases (Burdett, 1979; McKay, 1992; Sharpe and Mason, 1992; Simpson, 1990), in others it has not (Ritchie et al., 1985; van den

Driessche, 1992). Recently, it has been proposed that RGC relates to seedling performance, not directly, but by virtue of a correlation with cold hardiness or other types of stress resistance that directly affect performance (Cannell et al., 1990; Omi et al., 1994).

As RGC is affected by cold storage the appropriate time to assess RGC is just prior to planting (Ritchie and Dunlop, 1980). Generally RGC is measured over 2 or more weeks. A shorter (7 day) test has been used for lodgepole pine seedlings (Burdett, 1979). Regardless of the test used, there is a hiatus while the seedlings are tested, during which time the nursery manager is unsure if stock should be planted. This is a major drawback to the method.

Initially, researchers hypothesized that high RGC was related to carbohydrate status (Kruger and Trappe, 1967; Munns and Weir, 1981; Shihoya et al., 1966). However, this does not appear to be the case. Studies showing large changes in root starch concentrations have not indicated a relationship with RGC (Rose, 1992). Ritchie (1982) concluded that stored total non-structural carbohydrate in the roots were not related to RGC. Finally, root carbohydrate reserves and new root growth related poorly in Sitka spruce (*Picea sitchensis*) (Deans et al., 1990) and Douglas-fir (*Pseudotsuga menziesii*) seedlings (Cannell et al., 1990).

2.3. Root Viability

Desiccation and cold damage to root systems is insidious, not always manifesting itself through visible symptoms. Indeed, tops of seedlings with dead root systems often appear quite normal, providing no hint of the seedlings' inevitable post-planting failure. Nurseries often rely on RGC testing to detect such damage (Lindström, 1986).

At a cellular level, the activity of succinate dehydrogenase, an enzyme involved in the respiration cycle, can be used as an index of cell viability. The reduction of colorless triphenyl tetrazolium chloride (TTC), brought about by the action of the dehydrogenase can be quantified spectrophotometrically (Steponkus and Lanphear, 1967). The test can be completed in 2 days. Viability measured by TTC reduction represents metabolic activity potential, at the time of the test.

TTC reduction has been used in a number of studies. Lindström and Nyström (1987) found that viability measured as TTC reduction was useful as a quick method for estimating

cold injury of roots. Watanable et al. (1992) used TTC as a protoplast viability test. Parker (1952) used TTC reduction to determine the viability of conifer needles following desiccation. Joslin and Henderson (1984) found a linear correlation between TTC reduction and dry weight of live root tissue of white oak (*Querceus alba*). The proportion of living root tissue following freeze-induced dehydration damage of overwintering Scots pine (*Pinus sylvestris*) seedlings was also determined using TTC reduction (Sutinen et al., 1996).

2.4 Needle Morphology

Most conifer seedlings can be classified as having one of three needle morphologies: primary, secondary or mixed (Scagel et al., 1993). Primary needles are the first single needles produced by all species of pine. They are usually found on seedlings with a single terminal bud. Depending upon the species, primary needles may be replaced quickly with secondary needles or, in the case of lodgepole pine, may continue to develop until the end of the first growing season. Primary needles seldom persist on the seedling beyond the first year of outplanting. Secondary needles are the first true fascicle needles, and they originate in the axis of the primary needles. Development of secondary needles can be induced in the nursery using photoperiod. The development of secondary needles in lodgepole pine is accompanied by differences in bud morphology (multiple terminal buds) and larger root collar diameter. Lodgepole pine secondary needles are considered to be more drought resistant than are primary needles (Scagel et al., 1993). Seedlings with mixed morphology have a few secondary needles toward the tip of the stem but only a single terminal bud.

2.5 Chlorophylls

Many studies have shown that chlorophyll fluorescence is a sensitive and early indicator of damage to photosynthesis, and to the plant in general, resulting from stress such as freezing, chilling and drought (Hetherington and Öquist, 1988; Örgren and Öquist, 1985; Strand and Öquist, 1988). Variable chlorophyll fluorescence has also proven useful in assessing seedling health following cold storage: rapid reactivation of photosynthesis was

associated with high RGC scores, while delayed or incomplete photosynthetic reactivation was associated with low RGC (Vidaver et al., 1989).

Water deficit (as caused by frost or drought) reduces chlorophyll content (Beadle and Jarvis, 1977). Manter and Livingston (1995) found that measuring chlorophyll loss is a better method to detect lethal and sublethal freezing injury in intact, red spruce (*Picea rubens*) in comparison to measuring photosynthetic rate or electrolyte leakage.

2.6 Carbohydrates

Carbohydrates in the form of starch and other total non-structural sugars are the major form of energy reserves for most woody plants (Glerum, 1980). Of these, starch is the most abundant form of storage carbohydrate in most seedling tissue (Glerum, 1980). Of the soluble carbohydrates, sucrose is the major photosynthetic product, the principal transportable carbohydrate, and the main storage carbohydrate in the above-ground tissue of many plants. Raffinose has also been identified as a storage carbohydrate in Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) shoots and needles, particularly during dormancy (Aronsson et al., 1976). The hexose-reducing sugars, fructose and glucose, are commonly present in poplar (*Populus trichocarpa*) roots in higher concentrations than is sucrose (Bonicel et al., 1990).

Starch and other non-structural carbohydrates are used for plant maintenance in the winter, being the major substrates for respiration (Cannell et al., 1990), and for initial growth the following season (Omi et al., 1994). Both spring root growth and shoot elongation have been shown to depend on carbohydrate reserves. As such, poor accumulation can profoundly affect seedling performance the following year (Farmer, 1978; Little, 1974). It has been postulated that the importance of lifting and storage practices may be related to the availability of carbohydrate reserves (McCracken, 1979; Webb and Alther, 1980). Although shoots, needles and roots all store carbohydrate, it appears that depletion of needle storage reserves in particular decreases field survival and growth (Puttonen, 1986). Root reserve levels (total non-structural carbohydrate) also decrease during cold storage (Deans et al., 1990;

Ritchie, 1987; Ronco, 1973). This reduction may (Venn, 1980) or may not (Jiang et al., 1994) affect subsequent field survival and growth.

In addition to providing reserves for growth, stored carbohydrates function in a number of other capacities. Increases in soluble carbohydrate during the autumn parallel increases in cold hardiness (Egger et al., 1996). This is not simply correlative, as sugars are known to provide water stress tolerance (both freezing and drought stress) by binding water, and acting as osmotica (Levitt, 1980; Sutinen et al., 1996). Rapid conversion of starch to glucose in the root has been correlated with renewed root growth (Johnson-Flanagan and Owens, 1985a). This again suggests that the soluble carbohydrates act as osmotica, resulting in rapid uptake of water and cell expansion.

2.6.1. Root Carbohydrates

The highest concentration of carbohydrate reserves are usually found in root tissue (Glerum, 1980). These root reserves change dramatically throughout the year, decreasing rapidly with bud-break, and early vegetative and reproductive development, then increasing late in the growing season, usually after cessation of vegetative growth (Rose, 1992; Webb and Kilpatrick, 1993).

2.6.2 Needle Carbohydrates

There is an annual fluctuation in carbohydrates in conifer needles. Egger et al. (1996) reported that glucose and fructose (the predominant soluble carbohydrates) increase in spruce needles during the cold period, then decrease in a nearly linear manner until bud break. In contrast, sucrose reaches its highest concentration in spring and fall. Starch content decreases during fall and winter then reaches very high levels before bud break the next spring. This pre-bud break starch accumulation is a result of photosynthetic activity (Fischer and Höll, 1991; Webb and Kilpatrick, 1993). Raffinose and stachyose, which are involved in the acquisition of frost tolerance, decrease from high levels in February to very low levels at bud break (Egger et al., 1996). Needle soluble carbohydrates increase during cold storage (Chomba et al., 1992).

2.7 Growth Cycle

The annual growth cycle of conifers can be divided into active growth, dormancy and quiescence. During dormancy (fall and early winter) trees have no growth competence. Dormancy is induced when trees are exposed to short photoperiod and low chilling temperatures. A dormancy requirement must then be met. This requirement can vary for different conifer species from 1,000 hours to 2,000 hours at 6°C (Ritchie et al., 1985). After the completion of dormancy, the tree enters the quiescence phase. This quiescence phase is maintained by low temperature (below 6°C) in nature or under cold storage conditions. The trees have full growth competence and will grow under favourable growth conditions.

Although the true definition of dormancy relates to growth and bud development, seedling dormancy is often assessed on the basis of stress tolerance (Timmis, 1980). The more dormant a seedling is, the more stress tolerant it is, because its metabolic activity is very much reduced. Thus, physiological changes associated with the state of dormancy have been assessed as increases in cold hardiness (Colombo et al., 1984).

2.8 Chilling Requirement

Chilling is an important factor in the development of both stress resistance and dormancy in conifer seedlings. Seedlings must accumulate chilling hours in the fall in order for growth to cease and dormancy to be induced. In lodgepole pine, 280 hours of temperature below 6°C (Ritchie, 1987) are needed in order for growth to cease. An additional 220 hours are needed for the induction of freezing tolerance, followed by a further 420 hours for the dormancy requirement to be satisfied. Warm days reduce the chilling effects of the cold (Krugman and Stone, 1966). Very low temperature also may retard or even halt the physiological processes associated with chilling fulfillment (Taylor and Dumbroff, 1975).

Despite the obvious complexities a static model for chilling has proven to be useful in determining the time during which the seedlings are accumulating chilling degree hours, and thereby entering into dormancy (Hänninen, 1990). The conventional chilling model applied by foresters is based on a 6° or 8°C threshold that does not impose a lower limit to chilling and does not provide for reversal of chilling (Ritchie, 1989).

Another method that has been used to assess suitable date for lifting is to determine frost hardiness (Colombo et al., 1984). Survival to - 18°C has been related to storability and has been used as a predictor of suitable lifting dates (Burdett and Simpson, 1984; Camm et al., 1994). Once seedlings receive their chilling requirement for dormancy, they can be lifted for storage. Cold storage is a common practice in Canada, USA and Scandinavia, as it maintains seedlings in the quiescence state, extending it through to the time of planting (Ritchie, 1984).

2.9 Lifting and Cold Storage

Storage of planting stock at - 2°C for up to six months has became a part of many typical conifer seedling production schedules (van Eerden and Gates, 1990). It allows stock to be lifted under favourable nursery conditions and maintained in a quiescence state until required for outplanting.

The success of long-term storage depends, in part, on the physiological status of the seedlings at the time of lifting. While cold storage can help satisfy the chilling requirement needed to attain seedling dormancy (Omi et al., 1991a), storage can be unsuccessful if seedlings are lifted before they have achieved adequate resistance to the stresses of handling and cold storage (Daniels and Simpson, 1990). Similarly, if lifting is delayed too long seedling health appears to be negatively affected (Omi et al., 1991b). This could be explained by resumption of growth and the associated reduction in stress tolerance and losses of storage reserves.

Storage conditions also affect seedling health. Seedling quality may decline in dark storage because seedlings are no longer exposed to natural signals such as a photoperiod (Lavender and Wareing, 1972); the temperature may be too cold to adequately satisfy the chilling requirement (Omi et al., 1991a); and seedling may become desiccated, and lose frost and drought resistance (Ritchie, 1982; Ritchie, 1986). Furthermore, carbohydrate reserves decline during long-term storage (Cannell et al., 1990; Jiang et al., 1994). In white spruce, cold storage decreased total non-structural carbohydrates. More specifically, total soluble carbohydrates decreased and starch increased (Jiang et al., 1994). This decrease in soluble

carbohydrate reserves may lead to reduced root initiation, lower seedling survival, and poor growth at outplanting (Duryea and McClain, 1984; Puttonen, 1986). On the other hand, such decreases may not impact on subsequent survival and growth (Jiang et al., 1994).

Although the rate of cooling would be expected to impact on the physiological status of the seedling, there is little or no reference to this in literature. Slow thawing promotes respiration and loss of carbohydrate reserves, impairing the ability of seedlings to function effectively in water or nutrient uptake on removal from storage (Camm et al., 1995).

Currently, it is recommended that frozen container stock be slow thawed in the dark for 14 to 19 days at 2 to 3°C (Fraser et al., 1990). Unfortunately, if outplanting delays further extend the time until planting, the seedlings may lose cold hardiness and deplete carbohydrate reserves (Omi et al. 1994; Ronco, 1973). An alternative thawing regime, which overcomes the problem of outplanting delays, is fast thawing at 21°C in the dark for 2-3 hours. Camm et al. (1995) found that seedlings submitted to fast thawing broke bud 3.3 days later than did slow thawed stock and had greater frost hardiness at planting time.

2.10 Drought and Desiccation Stress Tolerance

Rapid, short term desiccation often occurs in conifer seedlings as they are moved from the nursery to the field (Ritchie et al., 1985; Tabbush, 1987) and drought stress often develops following outplanting. The latter is a result of poor water uptake by roots, and leads to high seedling mortality and retarded growth of surviving seedlings (Burdett, 1990). Therefore, successful establishment of newly planted seedlings depends partly on their drought tolerance (Blake and Sutton, 1987).

The ability of plants to tolerate drought is strongly influenced by their genotype and their life history (Seiler and Johnson, 1988). Sudden environmental stress is often lethal to plants that have never encountered adverse growing conditions. While some plants have an inherent potential for drought resistance, other must be exposed to limited drought in order to develop tolerance (Levitt, 1980). This acclimation process involves morphological and biochemical changes.

The physiological mechanisms by which conifer seedlings respond to drought and osmotic stress are poorly understood (Levitt, 1980). It remains unclear which changes reflect the onset of stress and which changes are associated with stress adjustment. Further, resistance to drought is mediated both by drought avoidance mechanisms that prevent the loss of water, through stomatal closure and rapid root extension, and by drought tolerance mechanisms that include solute accumulation and adjustment of photosynthesis to low water potentials.

Solute accumulation in drought-conditioned black spruce (*Picea marina*) was observed to contribute more than stomatal conductance in helping plants adapt to osmotic stress (Zwiazek and Blake, 1989). Sugars and amino acids are known to be major constituents of osmoregulation in black spruce. The increases in various monosaccharides, including mannose, fructose, galactose and glucose during one exposure to drought corresponded with a decline in sucrose (Zwiazek and Blake, 1990). Such hydrolysis of sucrose could benefit a stressed plant because one molecule of sucrose yields two osmotically active molecules (glucose and fructose) with minimal cost to the plant.

A recent study by Jiang et al. (1995) noted that high starch content rather than high soluble sugar content led to improved potential for drought stress tolerance in white spruce. They felt that the starch yielded more sugars during the drought and this in turn altered the osmotic potential in favour of reduced plasmolysis under stress conditions. In this case, tolerance was assessed on the basis of gas exchange rates and survival.

Changes in certain soluble carbohydrates may not be detected by measuring total soluble or total non-structural carbohydrates. As indicated above, increases in glucose and fructose were paralleled with decreases in sucrose. Thomas (1990) found that the total non-structural carbohydrate pool was not affected by drought conditioning, but a decrease in starch concentration in the roots suggested that some hydrolysis of starch to hexoses occurred.

Drought tolerance during outplanting can be induced by exposure of conifer seedlings to drought stress prior to cold storage and outplanting (Kaushal and Aussenec, 1989). Nursery drought stress cycles and low temperature increase lodgepole pine survival but reduce height and dry weight (van den Driessche, 1991). This indicates that drought

tolerance can be manipulated by drought treatments and temperature, however, the most effective treatments tend to produce the smallest seedlings. The long term effects of this are not known as large seedlings usually have higher survival and grow more quickly than do small seedlings when planted in a reforestation site (van den Driessche, 1984).

Nursery nutrient regimes are also known to influence drought tolerance in conifers. For example, fertilization with nitrogen has been shown to reduce drought stress in Douglas-fir (Brix and Michell, 1986). By appropriate nitrogen and drought treatments it was possible to increase lodgepole pine survival by 33% in a sand bed nursery (van den Driessche, 1992). Increased tissue potassium concentrations have also been shown to decrease transpiration and increase drought tolerance in conifer seedlings (Bradbury and Malcolm, 1977).

Blake (1983) found that cold storage of white spruce seedlings led to reduced water loss when water was limiting. This did not reduce stomatal opening and hence photosynthetic potential, when there was adequate moisture. Outplanted, cold-stored seedlings showed only slight changes in water potential and diffusive resistance. This was associated with minimum transplanting stress.

2.11 Electrolyte Leakage

Cell desiccation and resultant plasmolysis underlay all the effects of drought stress. Cells from tolerant tissues undergo less plasmolysis and hence have less membrane damage than do cells from non-tolerant tissues. This membrane damage can be measured as electrolyte leakage.

Results from Sitka spruce and Douglas-fir indicated a highly significant, negative relationship between fine-root electrolyte leakage and seedling field survival following storage (McKay and Mason, 1991). This suggests that fine-root leakage is an excellent, objective indicator of plant vitality following cold storage.

The close relationship between survival and root electrolyte leakage after cold storage suggests that deterioration of the fine-root system during storage is a major cause of plant failure. Burdett (1990) proposed a model of seedling establishment in which the water status

of the transplanted seedlings is crucial. In this model, a high water status allows photosynthesis to proceed, promoting new root growth, which increases water (and mineral) uptake, whereas a low plant moisture content inhibits photosynthesis, reducing root growth and exacerbating drought stress. One factor affecting water status immediately after planting is the ability of the existing root system to supply water.

This brings us back to drought tolerance. If the seedling is drought tolerant, the root system, with its higher osmotic potential, will be able to take up more water under stress conditions than its non-tolerant counterpart. In addition, the seedling will be better able to withstand the stress. Thus, measurement of percent electrolyte leakage following exposure to drought stress should provide important information about the health of seedlings prior to outplanting and their tolerance to drought stress following outplanting.

2.12 Literature Cited

Aronsson, A., Ingestad, V.E., and Volenec, J.J. 1976. Carbohydrate metabolism and frost hardiness in pine and spruce seedlings grown at different photoperiods and thermoperiods. Physiol. Plant. 36:127-132.

Beadle, C.L., and Jarvis, P.G. 1977. The effect of shoot water status on some photosynthetic partial processes in Sitka spruce. Physiol. Plant. 41:7-13.

Blake, R.J. 1983. Transplanting stress in white spruce: Effect of cold storage and root pruning on water relations and stomata conditioning. Physiol. Plant. 57:210-216.

Blake, T.J., and R.F. Sutton.1987. Variation in water relations of black spruce stock types planted in Ontario. Tree Physiol. 3:331-344.

Bonicel, A., and Vercosa de Medeiros Raposo, N. 1990. Variation of starch and soluble sugars in selected sections of poplar buds during dormancy and post-dormancy. Plant Physiol. Biochem. 28:577-586.

Bradbury, I.K., and D.C. Malcolm. 1977. The effect of phosphorus and potassium on transpiration, leaf diffusive resistance and water use efficiency in Sitka spruce (*Picea sitchensis*) seedlings. J. Appl. Ecol. 14:631-641.

Brix, H., and A.K. Mitchell. 1986. Thinning and nitrogen fertilization effects on soil and tree water stress in a Douglas-fir stand. Can. J. For Res. 16:1334-1338.

Burdett, A.N. 1979. New methods for measuring root growth capacity: their value in assessing lodgepole pine stock quality. Can. J. For. Res. 9:63-67.

Burdett, A.N. 1987. Understanding root growth capacity: theoretical consideration in assessing planting stock quality by means of root growth tests. Can. J. For. Res. 17:768-775.

Burdett, A.N. 1990. Physiological processes in plantation establishment and the development of specifications for forest planting stock. Can. J. For. Res. 20:415-427.

Burdett, A.N., and Simpson, D.G. 1984. Lifting, grading, packaging. *In* Forestry nursery manual: production of bareroot seedlings. Edited by M.L.Duryea and T.D.Landis. Forest Research Laboratory, Oregon State University, Corvallis, Nijhof/Junk, The Hague, Boston, pp. 227-234.

Camm, E.L., Goetze, D.C., Silim, S.N., and Lavender, D.P. 1994. Cold storage of conifer seedlings: An update from the British Columbia perspective. For. Chron. 70:311-316.

Camm, E.L., Guy, R.D., Kubien, D.S., Goetze, D.C., Silim, S.N., and Burton, P.J. 1995. Physiological recovery of freezer-stored white and Engelmann spruce seedlings planted following different thawing regimes. New For. 10:55-77.

Cannell, M.G.R., Tabbush, P.M., Deans, J.D., Hollingsworth, M.K., Sheppard, L.J., Philipson, J.J., and Murray, M.B. 1990. Sitka spruce and Douglas-fir seedlings in the nursery and in cold storage: root growth potential, carbohydrate content, dormancy, frost hardiness and mitotic index. Forestry 63:9-27.

Chomba, M.B., Guy, R.D., and Weger, H. 1993. Carbohydrate reserves accumulation and depletion in Engelmann spruce (*Picea engelmannii*, Parry): effect of storage and pre-storage CO_2 enrichment. Tree Physiol. 13:351-364.

Colombo S.J., Glerum, C., and Webb, D.P. 1984. Frost hardiness testing: an operational manual for use with extended greenhouse culture. Canadian Forest Service, Great Lakes Research Center, Sault Ste. Marie, Ontario. For. Res. Rep. No.110.

Daniels, T.G., and Simpson, D.G. 1990. Seedling production and processing: bareroot. *In* Regenerating British Columbia's forests. Edited by D.P. Lavender, R. Parish, C.M. Johnson, G. Montgomery, A. Vyse, R.A. Willis, and D. Winston. University of British Columbia Press, Vancouver. pp. 206-225.

Deans, J.D., Lundberg, C., Tabbush, P.M., Cannell, M.G.R., Sheppard, L.J., and Murray, M.B. 1990. The influence of desiccation, rough handling and cold storage on the quality and establishment of Sitka spruce planting stock. Forestry. 63:129-141.

Duryea, M.L., and McClain, K.M. 1984. Altering seedling physiology to improve reforestation success. *In* Seedling physiology and reforestation success. Edited by M.L. Duryea and G.N. Brown. Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands. pp. 77-114.

Egger, B., Eining, W., Schlereth, A., Wallenda, T., Magel, E., Loewl, A., and Hampp, R. 1996. Carbohydrate metabolism in one- and two-year-old spruce needles, and stem carbohydrates from three months before until three months after bud break. Physiol. Plant. 96:91-100.

Farmer, R.E.Jr. 1978. Seasonal carbohydrate levels in roots of Appalachian hardwood planting stock. Tree Plant. Notes 29(3):22-24.

Fischer, Ch., and Höll, W. 1991. Food reserves of Scots pine (*Pinus sylvestris* L.). Trees 5:187-195.

Fraser, B., Haywood-Farmer, S., and Kooistra, C. 1990. Thawing guidelines for freezer stock. *In* Consumer's Guide to Tree Seedlings. A workbook on Production, Testing and Handling. *Edited by* R. Scagel and R. Evans. Canada-British Columbia Forest Resources Development Agreement, Victoria, BC. pp. 61-64.

Glerum, C. 1980. Food sinks and food reserves of trees in temperate climates. N.Z.J. For. Sci. 10:176-185.

Hänninen, H. 1990. Modeling bud dormancy release in tree from cool and temperate regions. Acta For. Fenn. 213:47-53.

Hetherington, S.E., and Öquist, G. 1988. Monitoring chilling injury: a comparison of chlorophyll fluorescence measurements, post chilling growth and visible symptoms of injury in *Zea maize*. Physiol. Plant. 72:241-247.

Jiang, Y., Zwaizek, J.J., and Macdonald, S.E. 1994. Effects of prolonged cold storage on carbohydrate and protein content and field performance of white spruce bareroot seedlings. Can. J. For. Res. 24:1369-1375.

Jiang, Y., Macdonald, S.E., and Zwiazek, J.J. 1995. Effects of cold storage and water stress on water relations and gas exchange of white spruce (*Picea glauca*) seedlings. Tree Physiol. 15:267-273.

Johnson-Flanagan, A.M., and Owens, J.N. 1985a. Development of white spruce (*Picea glauca*) seedlings roots. Can. J. Bot. 63:456-462.

Johnson-Flanagan, A.M., and Owens, J. N. 1985b. Root growth and root growth capacity of white spruce (*Picea glauca* [Moench] Voss) seedlings. Can. J. For. Res. 15:625-630.

Johnson-Flanagan, A.M., and Owens, J. N. 1986. Root respiration in white spruce (*Picea glauca* [Moench] Voss) seedlings in relation to morphology and environment. Plant Physiol. 81:12-25.

Joslin, J.D., and Henderson, G.S. 1984. The determination of percentages of living tissue in woody fine root samples using triphenyltetrazolium chloride. For. Sci. 30:965-970.

Kaushal, P., and G. Aussenec. 1989. Transplanting shock in Corsian pine and cedar of Atlas seedlings: internal water deficits, growth and root regeneration. For. Ecol. Manage. 27:29-40.

Krueger, K.M., and Trappe, J.M. 1967. Food reserves and seasonal growth of Douglas-fir seedlings. For. Sci. 13:192-202.

Krugman, S.L., and Stone, E.C. 1966. The effect of cold nights on the root-regenerating potential of ponderosa pine seedlings. For. Sci. 12:451-459.

Lavender, D.P., and Wareing, P.F. 1972. Effects of daylights and chilling on the response of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings to root damage and storage. New Phytol. 71:1055-1067.

Levitt, J. 1980. Responses of plants to environmental stresses. Academic Press, New York, 497 pp.

Lindström, A. 1986. Freezing temperatures in the root zone-effects on growth of containerized Scots pine and Norway spruce seedlings. Scan. J. For. Res. 3:371-377.

Lindström, A., and Nyström, C. 1987. Seasonal variation in root hardiness of container-grown Scots pine, Norway spruce, and lodgepole pine seedlings. Can. J. For. Res. 17:787-793.

Little, C.H.A. 1974. Relationship between the starch level at bud break and current shoot growth in *Abies balsamea* L. Can. J. For. Res. 4:268-273.

Lyr, H., and Hoffman, G. 1972. Growth rates and growth periodicity of tree roots. In: International Review of Forestry Research.Edited by J.A. Romberger and P. Mikola, Vol. 2., Academic Press, N.Y, London, pp. 181-236.

Manter, D.K., and Livingston, W.H. 1995. Influence of thawing rate and fungal infection by *Rhizosphaera kallhoffii* on injury in red spruce (*Picea rubens*) needles. Can J. For. Res. 26:918-927.

McCraken, I.J. 1979. Changes in the carbohydrate concentration of pine seedlings after cool storage. N.Z.J. For. Sci. 9:34-43.

McKay, H.M. 1992. Electrolyte leakage from fine roots of conifer seedlings: a rapid index of plant viability following cold storage. Can. J. For. Res. 22:1371-1377.

McKay, H.M., and Mason, W.L. 1991. Physiological indicators of tolerance to cold storage in Sitka spuce and Douglas-fir seedlings. Can. J. For. Res. 21:890-901.

Munns, R., and Weir, R. 1981. Contribution of sugars to osmotic adjustment in elongated and expanded zones of wheat leaves during moderate water deficits at two light levels. Aus. J. Plant. Physiol. 8:93-105.

Ögren, E., and Öquist, G. 1985. Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition susceptibility in intact willow leaves. Planta. 166:380-388.

Omi, S.K., Rose, R., and Sabin, T. 1991a. Effectiveness of freezer storage in fulfilling the chilling requirement of fall-lifted ponderosa pine seedling. New Forests. 5:305-326.

Omi, S.K., Yoder, B., and Rose, R. 1991b. Fall lifting and long-term freezer storage of ponderosa pine seedlings: effects on post storage leaf water potential, stomatal conductance, and root growth potential. Tree Physiol. 8:315-325.

Omi, S.K., Rose, R., and Sabin, T.E. 1994. Fall lifting and long-term freezer storage of ponderosa pine seedlings: effects on starch, root growth, and field performance. Can. J. For. Res. 24:624-637.

Parker, J. 1952. Desiccation in conifer leaves. Anatomical changes and determination of the lethal level. Bot. Gaz. 114:189-198.

Puttonen, P. 1986. Carboyhdrate reserves in *Pinus sylvestris* seedling needles as an attribute of seedling vigor. Scand. J. For. Res. 1:181-193.

Ritchie, G.A. 1982. Carbohydrate concentration reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Can. J. For. Res. 12:905-912.

Ritchie, G.A. 1984. Effect of freezer storage on bud dormancy release in Douglas fir seedlings. Can. J. For. Res. 14:186-190.

Ritchie, G.A. 1986. Relationship among bud dormancy status, cold hardiness, and stress resistance in 2+0 Douglas fir. New For. 1:29-42.

Ritchie, G.A. 1987. Some effects of cold storage on seedling physiology. Tree Plant. Notes 38:11-15.

Ritchie, G.A. 1989. Integrated growing schedules for achieving physiological uniformity in coniferous planting stock. Forestry (Supp.), 62:213-226.

Ritchie, G.A., and Dunlop, J.R. 1980. Root growth potential: its development and expression in forest tree seedlings. N.Z.J. For. Sci. 10:218-248.

Ritchie, G.A., Roden, J.R., and Kleyn, N. 1985. Physiological quality of lodgepole pine and interior spruce seedlings: effects of lift date and duration of freezer storage. Can. J. For. Res. 15:636-645.

Ritchie, G.A., and Tanaka, Y. 1990. Root growth potential and the target seedling. *In* Target Seedling Symposium: Proceedings, Combined Meeting of the Western Forest Nursery Associations, 13-17 Aug. 1990. Roseburg. Oregon. *Edited by* R.Rose, S.J. Campell, and T.D Landis. U.S. For. Serv. Rocky Mt. For. Range EXp. Stn. Gen. Tech. Rep. RM-200. pp. 37-51.

Ronco, F. 1973. Food reserves of Engelmann spruce planting stock. For. Sci. 19:213-219.

Rose, R. 1992. Root growth potential and starch differences in seedlings of six families of genetically improved loblolly pine. For. Sci. 38:448-456.

Scagel, R., Bonden, R., Madill, M., and Kooistra, C. 1993. Provincial seedling stock type selection and ordering guideline. Province of British Columbia, Ministry of Forests, pp. 19-20.

Seiler, J.R., and Johnson, J.D. 1988. Physiological and morphological responses of three half-sib families of loblolly pine to water-stress conditioning. For. Sci. 34:487-495.

Sharpe, A.L., and Mason, W.L. 1992. Some methods of cold storage can seriously affect root growth potential and root moisture content and subsequent forest performance of Sitka spruce and Douglas fir transplants. Forestry 65:463-476.

Shiroya,T., Lister, G.R., Slankis, V., Krotkov, G., and Nelson, C.D. 1966. Seasonal changes in respiration, photosyntesis, and translocation of the ^{14}C-labelled products of photosynthesis in young *Pinus strobilus* L. plants. Ann. Bot. 30:81-91.

Simpson, S. 1990. Frost hardiness, root growth capacity, and field performance relationship in interior spruce, lodgepole pine, Douglas-fir and western hemlock seedlings. Can. J. For. Res. 20:566-572.

Strand, M., and Öquist, G. 1988. Effect of frost hardening, dehardening and freezing stress on *in vivo* chlorophyll fluorescence of seedlings of Scots pine (*Pinus sylvestris* L.). Plant Cell Environ. 11:231-238.

Steponkus, P.L., and Lanphear, F.O. 1967. Refinement of the triphenyl tetrazolium chloride method of determining cold injury. Plant Physiol. 42:1423-1426.

Stone, E.C., and Jenkinson, J.L. 1971. Physiological grading of ponderosa pine nursery stock. J. For. 69:31-33.

Stone, E.C., Jenkinson, J.L., and Krugman, S.L. 1962. Root-regenerating potential of Douglas-fir seedlings lifted at different times of the year. For. Sci. 8:288-297.

Sutcliffe, J.F., and Sexton, R. 1968. Cell differentiation in the root in relation to physiological function. *In* Root growth. Edited by W.J. Whittington. Proc. Easter Tch. Agric. Sci. Univ. Nottingham. No. 15. pp. 80-101.

Sutinen, M.L., Mäkitalo, K., and Sutinen, R. 1996. Freezing dehydration damages roots of containerized Scots pine (*Pinus sylvestris*) seedling overwintering under subarctic conditions. Can. J. For. Res. 26:1602-1609.

Tabbush, P.M. 1986. Rough handling, soil temperature, and root development in outplanted Sitka spruce and Douglas-fir. Can. J. For. Res. 16:1385-1388.

Tabbush, P.M. 1987. Effect of desiccation on water status and forest performance of bare-rooted Sitka spruce and Douglas fir transplants. Forestry 60:31-43.

Tabbush, P.M. 1988. Silvicultural practices for plant restocking. Fom. Comm. Bull. (U.K.) 76.

Taylor, J.S., and Dumbroff, E.B. 1975. Bud, root, and growth-regulator activity in *Acer saccharum* during the dormant season. Can. J. Bot. 53:321-331.

Thomas, H. 1990. Osmotic adjusment in *Lolium perene;* it heritability and the nature of solute accumulation. Ann. Bot. 66:521-530.

Timmis, R. 1980. Stress resistance and quality criteria for tree seedlings: analysis, measurement and use. N.Z.J. For. Sci. 10:21-53.

van den Driessche, R. 1984. Relationship between spacing and nitrogen fertilization of seedlings in nursery, seedling mineral nutrition, and outplanting performance. Can. J. For. Res. 14:431-436.

van den Driessche, R. 1988. Nursery growth of conifer seedlings using fertilizers of different solubilities and application time, and their forest growth. Can. J. For. Res. 18:172-180.

van den Driessche, R. 1991. Influence of container nursery regimes on drought resistance of seedlings following planting. I. Survival and growth. Can. J. For. Res. 21:555-565.

van den Driessche, R. 1992. Changes in drought resistance and root growth capacity of container seedlings in response to nursery drought, nitrogen, and potassium treatments. Can. J. For. Res. 22:740-749.

van Eerdan, E., and Gates, J.W. 1990. Seedling production and processing: Container. *In* Regenerating British Columbia's Forests. *Edited by* D.P. Lavender, R. Parish, C. Johnson, G. Montgomery, A. Vyse, R.A. Willes and D. Winston. Univ. of BC Press. Vancouver, BC. 372 pp.

Venn, K. 1980. Winter vigour in *Pices abies* (L.) Karst. VII. Development of injury to seedlings during over winter cold storage. A literature review. Rep. Norw. For. Res. Inst. 35.9.

Vidaver W., Binder W., Brooke R.C., Lister G.R., and Toivonen, P.M.A. 1989. Assessment of photosynthetic activity of nursery grown *Picea glauca* seedlings using an integrating fluorometer to monitor variable chlorophyll fluorescence. Can. J. For. Sci. 19:1478-1482.

Watanable, M., Watanable, Y., and Noritsugu, S. 1992. Colorimetric estimation of leaf-protoplast potential-to-divide by use of 2,3,5-triphenyl tetrazolium chloride. Plant. Physiol. 14:111-114.

Webb, W.L., and Kilpatrick, K.J. 1993. Starch content in Douglas-fir-diurnal and seasonal dynamics. For. Sci. 39:359-367.

Webb, D.P., and Althen, F.N. 1980. Storage of hardwood planting stock, effects of various storage regimes and packaging methods on root growth and physiological quality. N.Z.J. For. Sci. 10:83-96.

Wilcox, H. 1954. Primary organization of active and dormant roots of noble fir, *Abies procera*. Am. J. Bot. 41:812-821.

Wilcox, H. 1968. Morphological studies of the roots of red pine, *Pinus resinosa* . I. Growth characteristics and patterns of branching. Am. J. Bot. 55:247-254.

Zwiazek, J.J. and T.J. Blake. 1989. Effects of preconditioning on subsequent water relations, stomatal sensitivity, and photosynthesis in osmotically stressed black spruce. Can. J. Bot. 67:2240-2244.

Zwiazek, J.J. and T.J. Blake. 1990. Effects of preconditioning on carbohydrate and amino acid composition of osmotically stressed black spruce (*Picea mariana*) cuttings. Can. J. For. Res. 20:108-112.

CHAPTER III

ROOT GROWTH AND MORPHOLOGY, CARBOHYDRATE RESERVES AND FIELD PERFORMANCE OF LODGEPOLE PINE SEEDLINGS

3.1. Introduction

In the past, much of the seedling research focused on outplanting survival and the development of assessment methods to predict seedling survival after outplanting. The methods developed include RGC (Camm and Harper, 1991), testing for frost hardiness (Warrington and Rook, 1980), root relative moisture content (RMC) (Sharpe and Mason, 1992), root electrolyte leakage (McKay and Mason, 1991), viability by TTC reduction (Birgas and D'Aoust, 1993) and chlorophyll fluorescence (Vidaver et al., 1989). Of these, RGC and testing for frost hardiness have been adopted by the nursery industry.

More recently, the focus has changed and the industry has begun to recognize the need for methods that not only predict survival following outplanting, but also predict superior productivity. To date, no methods have been developed. It is unlikely that the above mentioned tests will be suitable, as they have already been shown to be inaccurate in their capacity as predictors of outplanting success (Burdett and Simpson, 1984; McKay and Mason, 1991; Ritchie and Dunlop, 1980; Sharpe and Mason, 1992).

The objective of the present study was to examine gross root morphology, root growth, TTC reduction, RGC and carbohydrate reserves in relation to seedling establishment (survival and growth) following outplanting. Their potential as rapid, direct indicators of lifting times that optimize seedling establishment was then assessed. I anticipated that carbohydrates would be superior to the other variables as predictors of seedling survival and growth potential after outplanting.

3.2 Materials and Methods

3.2.1 Plant Material

Pinus contorta Dougl. spp. *latifolia* (lodgepole pine; seedlot #30862: 1500 m elevation; 50°54'N; 121°08'W) was chosen for the investigation. The seeds were sown in soil mix (50% peat, 50% sawdust, 1360 g dolomite, 487 g micromax, 1.75 kg miniprill and 2.5 kg nutricote type 180) on March 20-22, 1995 (Eileen Brader per. comm.). The seedlings were grown in a greenhouse at Hybrid Nursery, Pitt Meadows, British Columbia. Throughout germination the temperature was maintained at 20°C day and night. Once seedcoats were off, (April 22) the temperature was lowered to 16°C and the photoperiod was extended to 20 hours using supplemental lights. By July 4, 95% of seedlings had secondary needles and a minimum height of 7 cm. Seedlings were kept comparatively dry and no supplemental lights were used after July 18. Seedlings were grown outside from August 10 until lifting. Standard watering and fertilization regimes for the interior British Columbia provenances were employed throughout the growing season ("Excel" forestry fertilizer, 100 ppm of soluble nitrogen supplemented with calcium, $MgSO_4$, copper and iron). Seedlings were shipped (refrigerated) overnight to Edmonton courtesy of Eileen Brader from Hybrid Nursery Ltd., Pitt Meadows, British Columbia.

3.2.2 Pre-storage Sampling

Samples of 60 seedlings were lifted from the nursery on September 19, October 16, and 31, November 14, December 01, and 12, 1995. Thirty seedlings were used for determination of total number of elongating, absorbing and brown roots on the whole root system. All roots studied were long laterals and were classified as elongating (white and lacking root hairs), absorbing (white and possessing root hairs), and brown (the root enveloped by a necrotic layer). After counting the number of roots on the whole root system, the roots were analyzed for reducing carbohydrate, total soluble carbohydrate and starch content. Of the remaining 30 seedlings, 10 were tested directly for viability with TTC, and 20 were used to assess RGC followed by viability assessment with TTC.

3.2.3 Cold Storage, Thawing Conditions and Post-Cold Storage Sampling

Samples of 60 seedlings were lifted on each date as above, and bundles of 10 seedlings were wrapped with cellophane around the root plugs, placed inside a waxed box containing a plastic-lined paper bag and stored at -2°C for 3-5.5 months. The seedlings were stored in the cold storage of Nursery Extension Services, BC Ministry of Forests, Surrey, BC. The air temperature inside the boxes in storage was monitored with data-loggers and reached the -2°C target temperature within 10 days of storage. The cold storage temperature was constantly monitored and remained at -2°C throughout the entire storage period. After storage, seedlings were shipped (refrigerated) overnight from Surrey to Edmonton. Shipping of seedlings was provided courtesy of Cheryl Calam. A sample of 60 seedlings for each lifting date was thawed at +4°C in a cold room for 7 days (see Table 3.1 for dates). The experiments outlined above were repeated.

3.2.4 Viability Testing (TTC)

Samples of 10 seedlings (before regrowth under RGC conditions) and 20 seedlings (after regrowth under RGC conditions) were submitted to viability testing following the method of Steponkus and Lanphear (1967). Roots were washed in cold tap water and the central root mass of each seedling was sampled by removing a 2 cm wide band of roots at a distance of approximately 1 cm from the bottom of the root plug. Samples (300 mg fresh weight) containing a mixture of all 3 root classes were put in 17 x 120 mm test tubes to which 6.0 mL of 0.6 % (w/v) triphenyl tetrazolium chloride (TTC) in 0.06 M (0.045M Na_2HPO_4 + 0.015M KH_2PO_4) and 0.05% (v/v) Tween 20 were added. Samples were incubated for 20 hours at room temperature. The TTC was drained and the tissue was rinsed once with distilled water. Tissue samples were extracted in 7 mL of 96% (v/v) ethanol in a water bath at 80°C for 10 minutes. The extracts were cooled and made to 10 mL final volume with 96% ethanol, then 1 mL was diluted with 2 mL of 96% ethanol. The absorbances were recorded at 520 nm on a Varian Techtron spectrophotometer model 635. Control roots (heat killed) were autoclaved for 15 minutes at 120°C prior to analysis (Lindström and Nyström, 1987). Viability of roots was calculated as:

$$\text{Viability } (\%) = \frac{Asr - Aar}{Awr - Aar} \times 100 \qquad \textit{(Equation 3.1)}$$

where:

Awr - absorbance at 520 nm of white roots (100% alive) obtained after 7 days regrowth under optimum conditions

Aar - absorbance at 520 nm of heat killed roots

Asr - absorbance at 520 nm of the sample roots

3.2.5 RGC Testing

Samples of 20 seedlings were individually transplanted into 95 by 230 mm pots containing sand:peat:vermiculite (2:1:1, v/v/v). Seedlings were grown for 7 days in a growth chamber at 22°C with a 16 hour photoperiod of 400 µmol $m^{-2}s^{-1}$ photon flux density and 75% relative humidity. Watering was to the point of runoff on days 1 and 4. RGC was expressed as the total number of white roots per seedling (Johnson-Flanagan and Owens, 1985). The number of white roots was monitored on three root plug regions of 5 cm each (top, middle and bottom).

3.2.6 Extraction of Carbohydrates

Root plugs of 30 seedlings were washed in tap water. Ten randomly selected seedling root plugs were separately used for collection of 50 mg fresh weight of each of elongating, absorbing, and brown roots. The samples included the root tip and up to 20 mm of tissue above the root tip. Root tissue was ground into a fine powder in liquid nitrogen with a mortar and pestle, extracted in 3.0 mL phosphate buffer (20 mM, pH 7.2), centrifuged at 2,200 rpm for 10 minutes and the supernatant analyzed for reducing and total soluble carbohydrate content (as discussed below).

3.2.6.1 Reducing Carbohydrate Content

An aliquot of the supernatant (0.5 mL) was mixed with 1.5 mL of 2,4-dinitrophenol reagent that was prepared by mixing 57.5 mL 5% NaOH containing 1.79 g dinitrophenol and 0.625 g phenol, with 125 mL deionized H_2O containing 25 g potassium sodium tartarate, and

diluting to 250 mL with deionized H_2O. The mixture was heated in a boiling water bath for 6 minutes, cooled for 3 minutes in running water (10°C) and the absorbance was read at 600 nm within 20 minutes (Ross, 1959). Reducing sugar concentration was calculated on the basis of a glucose standard curve represented by the equation:

$$y = -0.0113 + 2.0875x, \quad r^2 = 0.997 \qquad (Equation\ 3.2)$$

where:

y = absorbance at 600 nm

x = amount of glucose (mg)

3.2.6.2 Soluble Carbohydrate Content

An aliquot of the supernatant (0.05 mL) from above was diluted ten-fold with 0.45 mL of 20 mM phosphate buffer (pH 7.2) and mixed with 0.5 mL 5% phenol, to which 2.5 mL concentrated sulfuric acid was added slowly. Following mixing, the solution was cooled to room temperature and the absorbance read at 485 nm (Dubois et al., 1956). Soluble carbohydrate concentration was calculated on the basis of a glucose standard curve represented by the equation:

$$y = 0.0071 + 0.0155x, \quad r^2 = 0.974 \qquad (Equation\ 3.3)$$

where:

y = absorbance at 485 nm

x = amount of glucose (μg)

3.2.6.3 Starch Content

Using the same seedlings as described in 3.2.6, 250 mg fresh weight samples of root tissue were ground into a fine powder in liquid nitrogen with a mortar and pestle, extracted in 5.0 mL of methanol:chloroform:water (MCW) (12:5:3 v/v/v) for 5 minutes, vortexed, centrifuged at 2,200 rpm for 10 minutes and the supernatant was carefully removed by aspiration. The extraction was repeated three times. The remaining MCW was evaporated to dryness by placing the rack of open tubes in an oven at 50°C for 4 hours, after which 4.0 mL of 0.1 N NaOH was added to each tube. The solution was mixed by vortexing until the starch

pellet was suspended. The starch was solubilized in a 50°C oven for 30 minutes with occasional swirling. Five mL of 0.1 N acetic acid was added to adjust the pH to 5.1. One mL of enzyme solution containing 2U/mL of amyloglucosidase (Sigma, CAT # A-7420, from *Aspergillus niger*) and 400 U/mL of α-amylase (Sigma, CAT # A 6211, crude, from *Aspergillus oryzae*), that was purified using a Centricon-30 microconcentrator (Amicon), was added to each tube. The tubes were then covered and placed immediately into an incubation oven at 50°C and incubated for 24 hours. The samples were removed from the oven, mixed well and centrifuged at 2,200 rpm for 10 minutes. Aliquots (0.5 mL) were mixed with 5.0 mL o-dianisidine (0.005% in 0.1 M sodium phosphate buffer, pH 7.0) solution containing glucose oxidase (GOD, Sigma, CAT # G-6125, from *Aspergillus niger*) and peroxidase (POD, Sigma, CAT # P-8000, crude, from Horseradish) to yield a final concentration of 5U of GOD/mL and 1U of POD/mL (GOD/POD/o-dianisidine). The samples were placed in an ice bath and 1.0 mL of 75% sulfuric acid was added. Once the samples were cooled, they were vortexed and the absorbance was read at 525 nm (Rose et al., 1991). Starch concentration was calculated on the basis of a glucose standard curve represented by equation:

$$y = 0.0044 + 0.0139x, \quad r^2 = 0.999 \qquad \textit{(Equation 3.4)}$$

where:

y = absorbance at 525 nm

x = amount of glucose (μg)

3.2.6.4 Quantification

The content of starch and reducing, total soluble, and total non-structural carbohydrates prior to and after storage was estimated for each root class. To calculate totals per root system these values were adjusted for the proportion of roots in each root class. Total non-structural carbohydrate content in different root classes, prior to and after storage, was estimated as the sum of the mean total soluble carbohydrate content plus mean starch content.

3.2.7 Outplanting

The seedlings (180 per lifting date) were removed from cold storage, shipped to Kelowna, BC, and thawed for 7 days at + 4°C according to the schedule outlined in Table 3.1. The seedlings were randomly planted on an uncultivated site, block 849-8 by Bear Creek, Kelowna (British Columbia) at 1340 m elevation (50°02'25"N; 119°40'05"W). Seedlings were outplanted at a 3 m x 3 m spacing over an area of approximately 1 ha. The site is located in the dry moderate subzone of the Montane spruce bioclimatic zone. The soil was a Luvisol with a texture of sandy clay loam. The site had a 7% slope with NE aspect and was neither irrigated nor weed controlled after planting. The site was provided courtesy of Kelly Fay from Riverside Forest Products, Kelowna, BC.

Table 3.1: Lifting and outplanting dates.

Lifting dates	Days between lifting and assessment	Assessment dates	Days between assessment and outplanting	Outplanting dates
Sep. 19, 1995	167	March 03, 1996	80	May 22, 1996
Oct. 16, 1995	138	March 03, 1996	80	May 22, 1996
Oct. 31, 1995	137	March 17, 1996	80	June 05, 1996
Nov. 14, 1995	123	March 17, 1996	80	June 05, 1996
Dec. 01, 1995	121	March 31, 1996	80	June 19, 1996
Dec. 12, 1995	109	March 31, 1996	80	June 19, 1996

The climate data (max/min temperature, sunshine hours, precipitation, relative humidity) for around the outplanting time (May 01 - July 31, 1996) were provided courtesy of Alan Nourse from Environment Canada, Mountain Weather Services Office, Kelowna, BC (Appendices I, II). The transformed climate data for the planting period were provided courtesy of Rob Scagel from Pacific Phytometric, Surrey, BC (Appendix III).

The 180 seedlings were assessed for survival at the end of the first growing season (October 06, 1996) and the number of dead seedlings recorded. At the same time, distal most

needle length (cm), collar diameter (mm), height at planting (cm), height increment in 1996 (cm) and total height (cm) were measured, and structure of the apical bud at planting and at the end of the growing season (mono/multi podial) and needle morphology (primary, secondary or mixed) were determined on 30 surviving seedlings for each lifting date.

3.2.8 Statistical Analysis

Simple linear correlations were computed to examine the relationships between field performance and root morphological parameters, (number of elongating, absorbing, brown roots, percent of white roots), viability before RGC, viability following RGC, RGC and carbohydrate pool (reducing, total soluble, total non-structural carbohydrates and starch) prior to and after storage. The mean value for each lifting date was used. In order to be considered for further statistical analyses, the parameters had to be correlated with field survival and/or performance. This potentially restricted the analyses to differences in percent of white roots, viability (TTC reduction), RGC and total root carbohydrates over six lifting dates. However, further statistical analyses for carbohydrates could not be carried out as the correlations were based on calculated values.

Differences in percent of white roots prior to and after cold storage, viability (TTC reduction) before and after RGC both prior to and after cold storage, RGC prior to and after cold storage over 6 lifting dates were determined with sources of variation of least difference (LD) and experimental error using one-way ANOVA. When the ANOVA showed a significant result ($p<0.05$), specific differences among LD means were determined with the least significant difference (LSD) test. All parameters listed above were analyzed by two-way ANOVA (lifting dates versus treatments - prior to and after cold storage). A significance level of $p<0.05$ was used. All statistical analyses were performed using the General Linear Model of SAS 6.10. program (SAS Institute Inc., Carry, NC 27513, USA).

3.3 Results

3.3.1 Root Morphology

The number of roots in each class; elongating, absorbing and brown, were monitored on the root system at each lifting date (Fig. 3.1a). On all dates, the root system had a very large percentage of white roots (sum of elongating and absorbing roots) (Fig. 3.1b). The percentage of white roots gradually increased between Sep. 19 and Nov. 14, when 90% of the roots were white (23% elongating and 76% absorbing). The percentage of white roots decreased thereafter. The changes noted arose from parallel increases and decreases in the absorbing and elongating roots (Fig. 3.1a). Seedlings lifted on Nov. 14 (Fig. 3.1b) had a significantly higher proportion of white roots (LSD, $p<0.05$) relative to those lifted on other dates (Appendix IV).

The distribution of white roots in the root plug after 7 days of regrowth in optimal conditions (RGC) is presented in Fig. 3.1c. White roots were located predominantly at the bottom of the root plug. There was a slight decrease in the proportion of white roots in the top region between Sep. 19 and Nov. 14, followed by a slight increase thereafter.

Following cold storage, the number of roots in each class was again monitored (Fig. 3.2a). There were no elongating roots. On all dates, there was a low percentage of white roots relative to that measured prior to cold storage (compare Fig. 3.1b with Fig. 3.2b). The percentage of white roots increased from Sep. 19 to Nov. 14, and decreased thereafter. Once again, the highest number of white roots after regrowth was located in the bottom of the root plug (Fig. 3.2c).

Viability (TTC reduction) results measured on seedling roots are presented in Fig. 3.3a. Root viability values were greater prior to cold storage than after cold storage on all dates except for Sep. 19. Root viability increased from Sep. 19 to Nov. 14 and decreased thereafter. There was a significant interaction between TTC reduction prior to and after cold storage and lifting date ($p<0.0001$) (Appendix IV).

Viability was examined after regrowth (when there would be more white roots) (Fig. 3.3b). Both before and after cold storage, viability was higher following regrowth on all dates

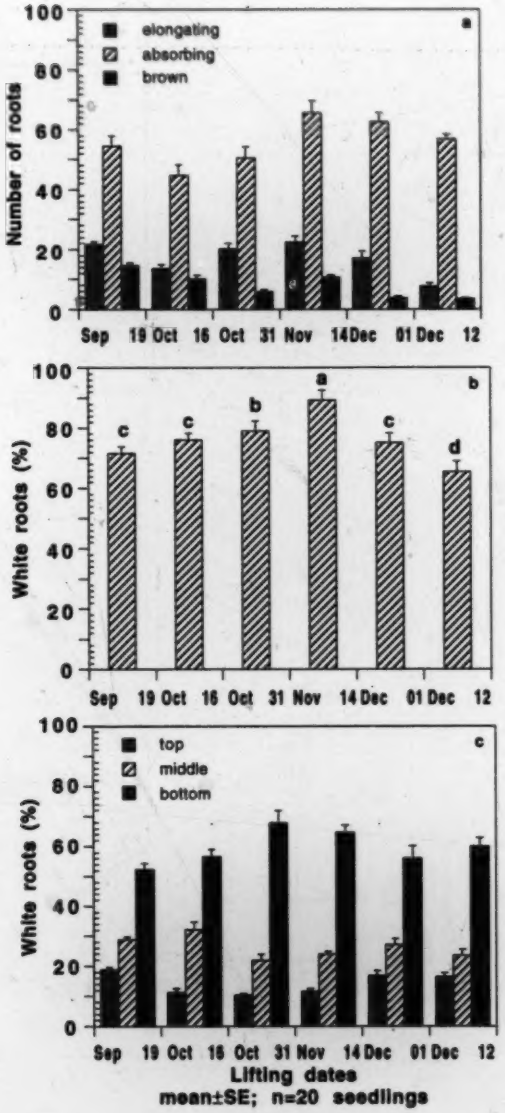


Figure 3.1: Root morphology data for lodgepole pine seedlings prior to cold storage

a) Number of elongating, absorbing and brown roots. Data were not analyzed by ANOVA.
b) Percent of white roots (sum of elongating and absorbing). Means having common letter(s) are not significantly different ($p>0.05$) according to a one-way ANOVA and LSD test.
c) Percent of white roots in different regions of the root system following RGC test. Data were not analyzed by ANOVA.

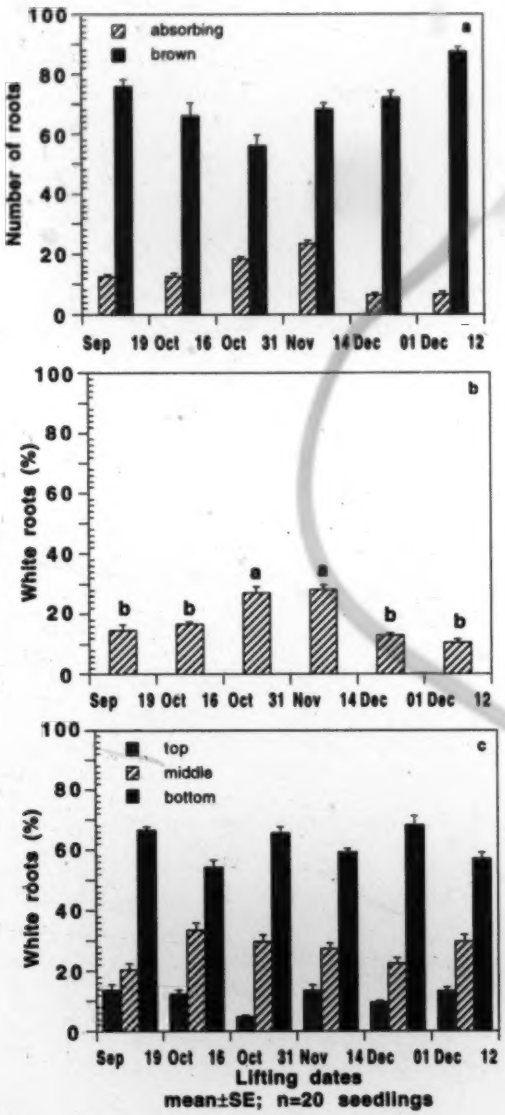


Figure 3.2: Root morphology data for lodgepole pine seedlings after cold storage

a) Number of absorbing and brown roots. Data were not analyzed by ANOVA.
b) Percent of white roots (sum of elongating and absorbing). Means having common letter(s) are not significantly different ($p>0.05$) according to a one-way ANOVA and LSD test.
c) Percent of white roots in different regions of the root system following RGC test. Data were not analyzed by ANOVA.

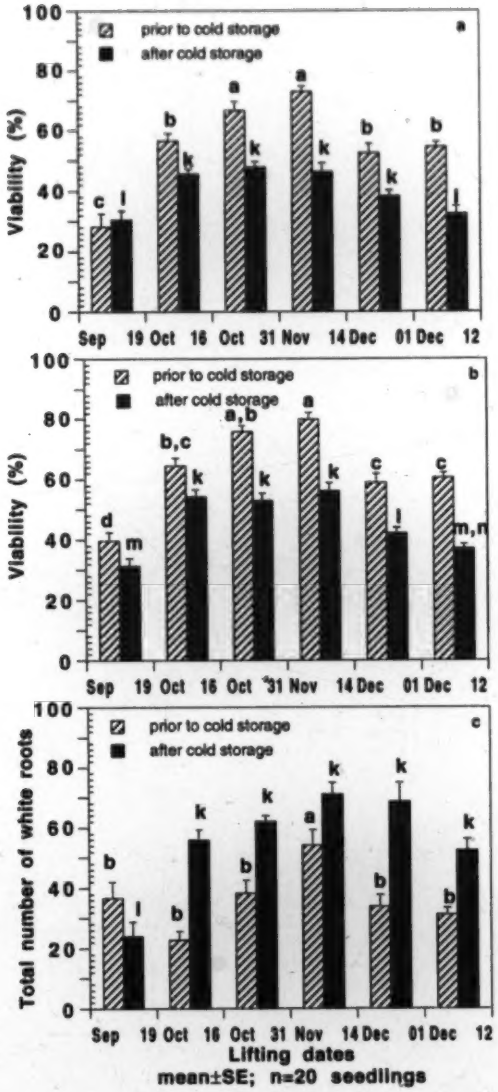


Figure 3.3: Viability and RGC for lodgepole pine seedlings prior to and after cold storage

a) Viability before RGC prior to and after cold storage measured as TTC reduction. Means having common letter(s) are not significantly different ($p>0.05$) prior to cold storage (a, b, c) and after cold storage (k, l, m) according to a one-way ANOVA and LSD test.
b) Viability after RGC prior to and after cold storage measured as TTC reduction. Statistics are as described for Fig. 3.3a.
c) RGC prior to and after cold storage. Statistics are as described for Fig. 3.3a.

except Sep. 19. There was a significant interaction between TTC reduction prior to and after cold storage and lifting date ($p<0.002$).

The relationship between root viability following regrowth, measured by TTC reduction, and RGC measured as number of white roots was assessed in roots prior to and after cold storage. Prior to cold storage there was no correlation between these two parameters ($n=20$, $r^2 = 0.18$, $p>0.4$). After cold storage there was a good correlation between RGC and viability ($n=20$, $r^2 = 0.64$, $p<0.05$).

RGC prior to and after storage is shown in Fig. 3.3c. The trend prior to cold storage was the same as the trend in white roots (Fig. 3.1b). Seedlings lifted on Nov. 14 had significantly higher RGC (LSD, $p<0.05$) than those from any other dates. There was no significant difference between samples on the other dates. RGC values were significantly higher (LSD, $p<0.05$) following cold storage than those prior to cold storage on all dates except for Sep.19. There was a significant interaction between RGC prior to and after cold storage and lifting date ($p<0.0001$).

3.3.2 Root Carbohydrates

There was little difference in the trend of reducing carbohydrates in the different root classes over the study period, prior to and after cold storage (Fig. 3.4a,b). There was a significant interaction between reducing carbohydrates in the root classes and lifting date prior to cold storage ($p<0.0005$). By combining the data for the root classes, a peak can be seen on Nov. 14, both prior to and after cold storage (Fig. 3.4c). No significant interaction occurred between reducing carbohydrates in root classes and lifting date following cold storage ($p<0.56$). Comparisons between total reducing carbohydrates prior to and after storage showed that, with the exception of Sep. 19, seedling roots appeared to have higher reducing carbohydrate contents after cold storage (Fig. 3.4c). In addition, the reducing carbohydrate contents varied more between sample dates after cold storage than before cold storage.

Total soluble carbohydrates content prior to cold storage was determined for all three root classes (Fig. 3.5a). Little variation was noted. No significant interaction occurred between

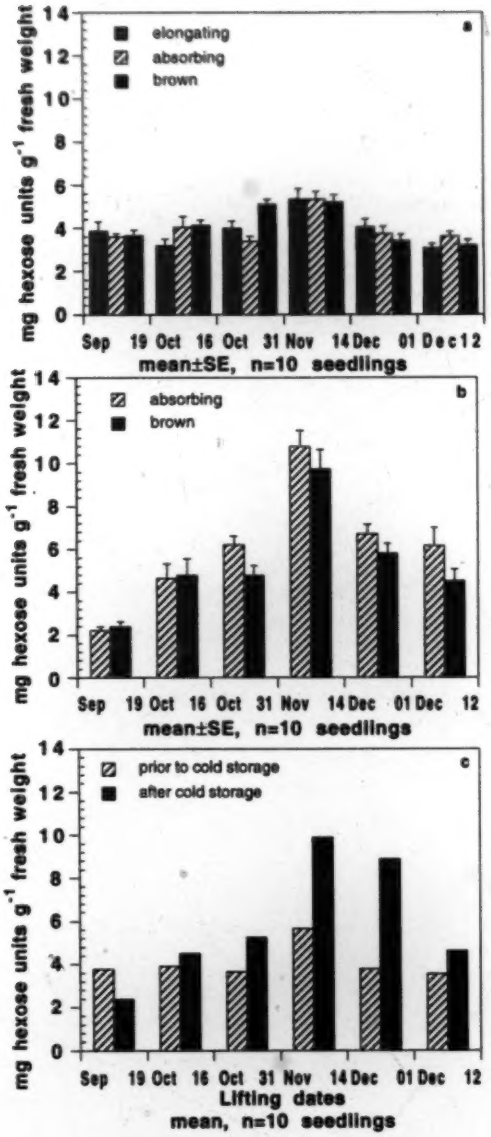

a
elongating
absorbing
brown
mg hexose units g-1 fresh weight
Sep 19 Oct 16 Oct 31 Nov 14 Dec 01 Dec 12
mean±SE, n=10 seedlings
b
absorbing
brown
mg hexose units g-1 fresh weight
Sep 19 Oct 16 Oct 31 Nov 14 Dec 01 Dec 12
mean±SE, n=10 seedlings
c
prior to cold storage
after cold storage
mg hexose units g-1 fresh weight
Sep 19 Oct 16 Oct 31 Nov 14 Dec 01 Dec 12
Lifting dates
mean, n=10 seedlings

Figure 3.4: Content of reducing carbohydrates in roots of lodgepole pine seedlings

a) In different root classes prior to cold storage. Data were not analyzed by ANOVA.
b) In different root classes after cold storage. Data were not analyzed by ANOVA.
c) In the root system prior to and after cold storage. Data are derived as described in materials and methods section. Data were not analyzed by ANOVA.

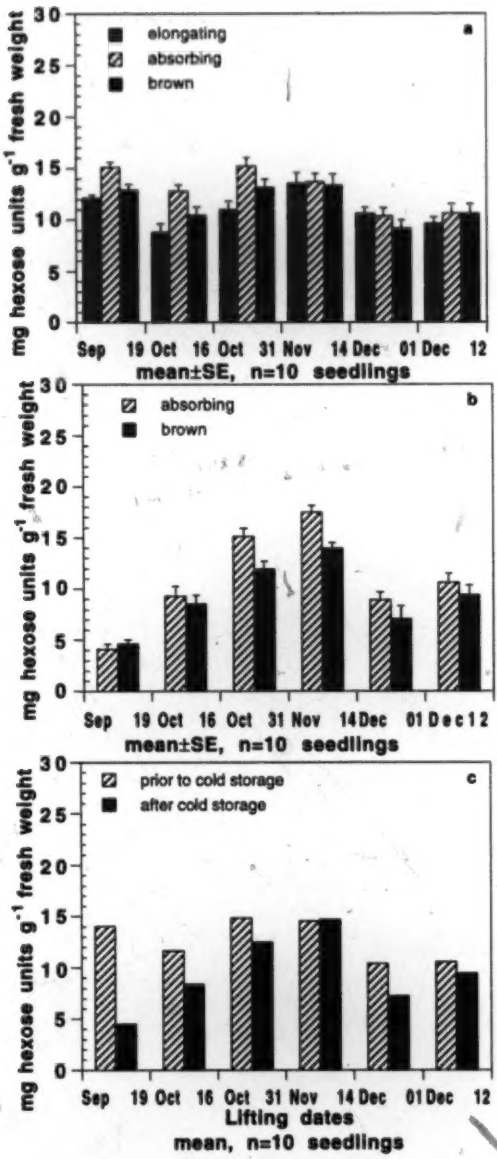


Figure 3.5: Content of total soluble carbohydrate in roots of lodgepole pine seedlings

a) In different root classes prior to cold storage. Data were not analyzed by ANOVA.
b) In different root classes after cold storage. Data were not analyzed by ANOVA.
c) In the root system prior to and after cold storage. Data are derived as described in materials and methods section. Data were not analyzed by ANOVA.

total soluble carbohydrates in root classes and lifting date prior to cold storage ($p<0.06$). Following cold storage, total soluble carbohydrates showed the same trend in absorbing and brown roots, increasing from Sep. 19 to Nov. 14, and decreasing thereafter (Fig. 3.5b). No significant interaction occurred between total soluble carbohydrates in root classes and lifting dates following cold storage ($p<0.14$). Comparisons between before and after cold storage data showed that storage appeared to be associated with a reduction in total soluble carbohydrates for all dates except Nov. 14 (Fig. 3.5c). The greatest decrease was measured in the Sep. 19 samples.

Starch content of seedlings prior to cold storage was relatively stable from Oct. 31 to Dec. 12 for all three root classes (Fig. 3.6a). In elongating roots the amount of starch was lower on Sep. 19, Oct. 16 and Dec. 12 than it was on other dates. there was a significant interaction between starch content in root classes and lifting date prior to cold storage ($p<0.0001$). Cold storage caused a dramatic decrease in starch content in both absorbing and brown roots (Fig. 3.6b). The effect of storage on starch content can be seen clearly in Figure 3.6c. The greatest decrease was measured in the Sep. 19 samples. Following cold storage, there was no significant interaction between starch content in root classes and lifting date ($p>0.98$).

Total non-structural carbohydrate was calculated as the sum of total soluble carbohydrate and starch. Throughout the study period, the absorbing roots had higher mean amounts of total non-structural carbohydrate than elongating and brown roots (Fig. 3.7a). On the first two sampling dates, Sep. 19 and Oct. 16, the elongating roots had less total non-structural carbohydrate than was measured over the remainder of the study. There was a significant interaction between total non-structural carbohydrates in root classes and lifting date prior to cold storage ($p<0.0003$).

After cold storage, the absorbing roots again had higher total non-structural carbohydrates in comparison with the brown roots, with the exception of the Sep. 19 samples (Fig. 3.7b). The total non-structural carbohydrate content decreased following storage, with the largest decrease in the Sep. 19 samples (Fig. 3.7c). The Nov. 14 samples lost the least total

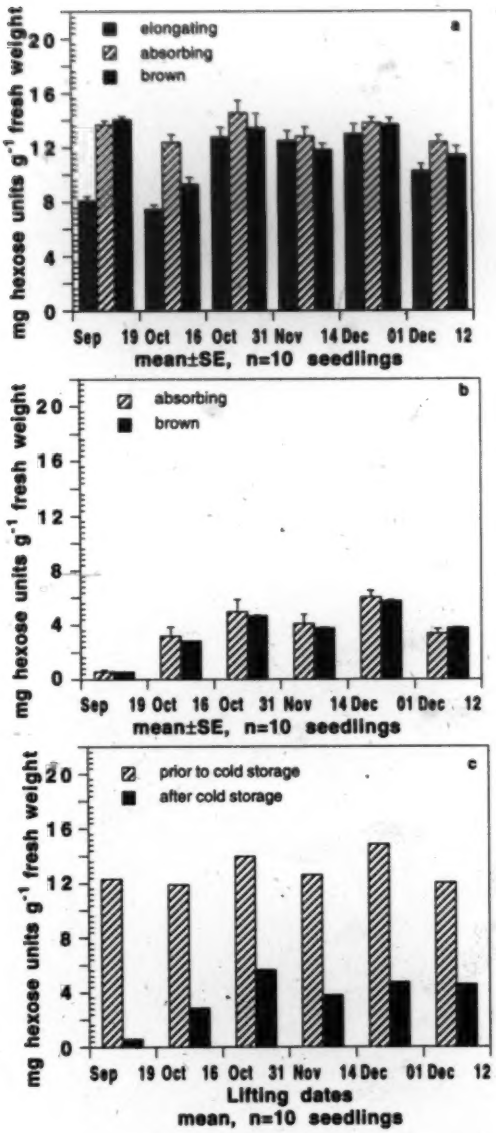


Figure 3.6: Starch content in roots of lodgepole pine seedlings

a) In different root classes prior to cold storage. Data were not analyzed by ANOVA.
b) In different root classes after cold storage. Data were not analyzed by ANOVA.
c) In the root system prior to and after cold storage. Data are derived as described in materials and methods section. Data were not analyzed by ANOVA.

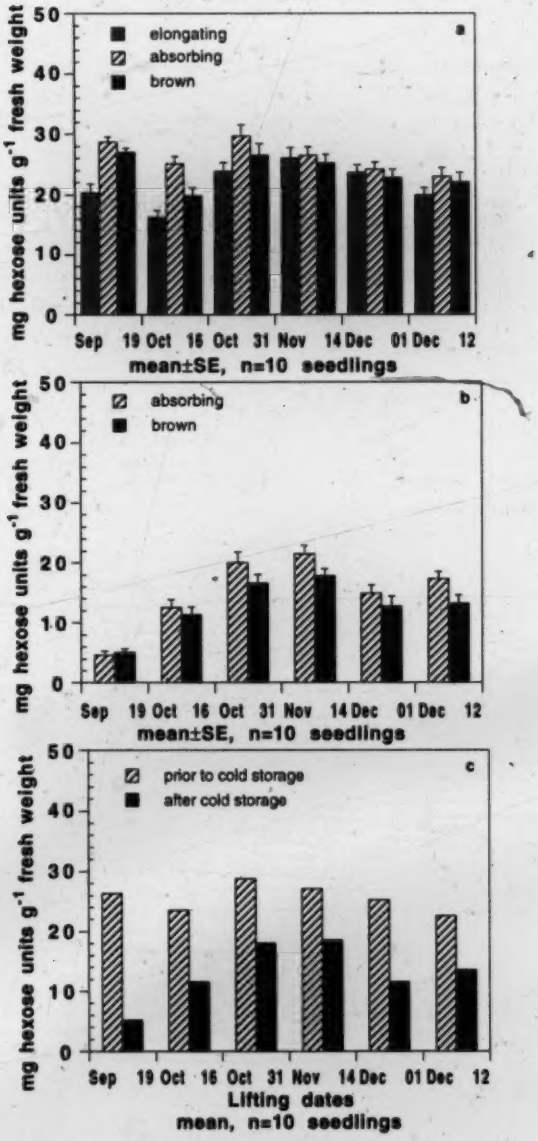


Figure 3.7: Content of total non-structural carbohydrate in roots of lodgepole pine seedlings

a) In different root classes prior to cold storage. Data were not analyzed by ANOVA.
b) In different root classes after cold storage. Data were not analyzed by ANOVA.
c) In the root system prior to and after cold storage. Data are derived as described in materials and methods section. Data were not analyzed by ANOVA.

non-structural carbohydrates during storage and consequently had the highest total non-structural carbohydrate in comparison with that measured on the other dates. Following cold storage, no significant interaction occurred between total non-structural carbohydrates in root classes and lifting date ($p<>0.32$).

In the present study, correlations between the various carbohydrates were examined. Correlation coefficients between reducing, total soluble, and total non-structural carbohydrates, starch and RGC, prior to and after cold storage are given in Table 3.2. As total non-structural carbohydrates were calculated as the sum of starch and total soluble carbohydrates, strong correlations would be anticipated. This was the case after cold storage, but prior to cold storage only total soluble carbohydrates were significantly correlated with total non-structural carbohydrates. RGC was significantly correlated with reducing carbohydrate prior to cold storage and with total soluble carbohydrates, total non-structural carbohydrates and starch after cold storage. The correlations were performed again using data from both 1995 and 1996 to determine whether correlations could be strengthened by increasing the sample size (see Chapter IV). This approach eliminated correlations with RGC after cold storage (Table 3.3).

3.3.3 Survival and Growth Parameters After the First Growing Season

Both the height at planting and the total height of seedlings at the end the growing season increased with later lifting (Table 3.4). Despite this, seedlings lifted on Nov. 14 showed the greatest height increment in 1996. Seedlings lifted on Nov. 14 also had the highest survival rate, largest collar diameter, the longest distal needles and the greatest height increment. There were no trends noted in needle morphology or apical bud morphology at planting or at the end of the growing season.

Climatic data (daily max/min temperatures, sunshine, precipitation and relative humidity and the 10 day weather summary after planting are shown in Appendices I, II and III. According to the data, none of seedlings were exposed to adverse conditions in the 10 days following outplanting.

Table 3.2: Correlation coefficients (r) for reducing carbohydrate, total soluble carbohydrate, starch, total non-structural carbohydrate and RGC prior to and after cold storage in 1995.

	Prior to Cold Storage				
	Reducing carbohydrate	**Total soluble carbohydrate**	**Starch**	**Total non-structural carbohydrate**	**RGC**
Reducing carbohydrate		0.44 (0.4)	-0.15 (0.8)	0.31 (0.5)	0.82 (0.04)
Total soluble carbohydrate	0.64 (0.2)		-0.06 (0.9)	0.86 (0.03)	0.62 (0.2)
Starch	0.54 (0.3)	0.66 (0.1)		0.47 (0.3)	-0.13 (0.8)
Total non-structural carbohydrate	0.62 (0.2)	0.97 (0.001)	0.82 (0.05)		0.48 (0.3)
RGC	0.71 (0.1)	0.83 (0.04)	0.84 (0.04)	0.89 (0.02)	
	After Cold Storage				

p values are indicated in brackets; n=6

Table 3.3: Correlation coefficients (r) for reducing carbohydrate, total soluble carbohydrate, starch, total non-structural carbohydrate and RGC prior to and after cold storage, in 1995 and 1996.

	Reducing carbohydrate	Total soluble carbohydrate	Starch	Total non-structural carbohydrate	RGC
Prior to Cold Storage					
Reducing carbohydrate		0.02 (0.9)	0.08 (0.1)	0.08 (0.8)	0.84 (0.01)
Total soluble carbohydrate	0.45 (0.2)		0.64 (0.9)	0.87 (0.001)	-0.06 (0.8)
Starch	0.52 (0.1)	0.54 (0.1)		0.93 (0.001)	-0.31 (0.4)
Total non-structural carbohydrate	0.50 (0.2)	0.93 (0.001)	0.80 (0.01)		-0.22 (0.6)
RGC	0.46 (0.2)	0.36 (0.3)	0.51 (0.2)	0.45 (0.2)	
After Cold Storage					

p values are indicated in brackets; n=9

Table 3.4: Field results of 1995 crop outplanted in spring of 1996.

Lifting date	Survival (%)	Collar diameter (mm)	Distal most needle length (cm)	Height at planting (cm)	Height increment (cm)	Total height (cm)	Apical bud (%)	Bud at planting (%)	Needle morphology (%)
September 19	13*	3.3 ± 0.1** [d]	3 ± 1.5[c]	7.9 ± 0.9[d]	5.8 ± 0.6[c]	13 ± 1.1[c]	47 mono-podial 53 multi-podial	29 mono-podial 71 multi-podial	53 primary 17 secondary 30 mixed***
October 16	90	4.3 ± 0.1[c]	4 ± 0.2[b]	10 ± 0.8[c]	11 ± 3[a,b]	21 ± 0.9[b]	16 mono-podial 84 multi-podial	13 mono-podial 87 multi-podial	3 primary 17 secondary 80 mixed
October 31	91	4.4 ± 0.2[b,c]	4.2 ± 0.2[a,b]	10 ± 0.5[c]	11 ± 0.7[a,b]	21 ± 0.9[b]	23 mono-podial 74 multi-podial	10 mono-podial 90 multi-podial	3 primary 20 secondary 77 mixed
November 14	95	5.1 ± 0.1[a]	5 ± 0.2[a]	12 ± 0.6[b,c]	12 ± 0.6[a]	24 ± 0.7[a]	7 mono-podial 93 multi-podial	19 mono-podial 81 multi-podial	30 primary 33 secondary 47 mixed
December 01	88	4.6 ± 0.2[b]	4.7 ± 0.3[a,b]	15.3 ± 1[a]	9.6 ± 0.6[b]	25 ± 0.7[a]	16 mono-podial 84 multi-podial	16 mono-podial 84 multi-podial	16 primary 26 secondary 58 mixed
December 12	86	4.6 ± 0.2[b,c]	4.7 ± 0.3[a,b]	14.8 ± 0.8[a,b]	9.9 ± 0.5[b]	25 ± 0.9[a]	30 mono-podial 70 multi-podial	3 mono-podial 97 multi-podial	16 primary 30 secondary 54 mixed

Mean ± SE, * n=180 seedlings for survival;**n=30 seedlings for all other parameters; ***Most primary needles were chlorotic
Means within a column having common letter(s) are not significantly different at p>0.05.

Correlation coefficients between field performance and both root morphological parameters and root growth indicators are given in Table 3.5. Surprisingly, the number of brown roots prior to storage was significantly correlated in an inverse manner with distal needle length, height at planting and total height. RGC is considered to be a good measure of survival. The results show that RGC after cold storage was significantly correlated with all measures of field performance and was not correlated with height at planting. Viability, measured as TTC reduction, before cold storage was a good predictor of field performance. Survival, collar diameter and height increment were all significantly correlated with viability. TTC reduction measured after cold storage significantly correlated only with height increment.

In order for a predictor to have general applicability for screening purposes, the regression equations from different data sets must be similar (McKay, 1992). This could not be addressed for RGC as there was only one data set for 1995. Following field assessment of the 1996 seedlings, the applicability of the RGC test will be reassessed. Results presented in Table 3.6 show that the slope of regression equations for TTC reduction are, in general, similar.

Correlation coefficients between carbohydrates and field performance are given in Table 3.7. No variables measured prior to storage correlated with field performance although total soluble carbohydrate was reasonably good. Following storage, total soluble carbohydrate showed a significant correlation with collar diameter, while starch was significantly correlated with survival, total height and distal needle length. Total non-structural carbohydrate correlated with survival and collar diameter.

3.4 Discussion

Assessment of the seedlings prior to cold storage suggested that seedlings lifted on Nov. 14 would have the greatest potential for outplanting success and subsequent tree growth. The seedlings had the highest number of white roots, the highest RGC and the highest number of absorbing roots.

Table 3.5: Correlation coefficients (r) for root morphological parameters with field performance.

Parameter		Survival	Collar diameter	Most distal needle length	Height at planting	Total height	Height increment
Number of elongating roots	*	- 0.33 (0.5)	- 0.20 (0.7)	- 0.43 (0.4)	- 0.54 (0.3)	- 0.45 (0.4)	- 0.25 (0.6)
	**						
Number of absorbing roots	*	0.11 (0.8)	0.49 (0.3)	0.47 (0.3)	0.55 (0.3)	0.41 (0.4)	- 0.22 (0.7)
	**	0.15 (0.8)	0.25 (0.6)	- 0.12 (0.8)	- 0.47 (0.3)	- 0.11 (0.8)	0.32 (0.5)
Number of brown roots	*	- 0.67 (0.1)	- 0.57 (0.2)	- 0.81 (0.05)	- 0.83 (0.05)	- 0.81 (0.05)	- 0.27 (0.6)
	**	- 0.28 (0.6)	- 0.04 (0.9)	0.01 (0.8)	0.42 (0.4)	0.09 (0.9)	- 0.42 (0.4)
% of white roots	*	0.36 (0.5)	0.45 (0.4)	0.15 (0.7)	- 0.15 (0.7)	0.16 (0.8)	0.45 (0.4)
	**	0.31 (0.5)	0.35 (0.5)	0.03 (0.9)	- 0.33 (0.5)	0.04 (0.9)	0.39 (0.4)
Viability before regrowth	*	0.99 (0.001)	0.91 (0.01)	0.71 (0.1)	0.36 (0.5)	0.74 (0.1)	0.81 (0.05)
	**	0.66 (0.2)	0.40 (0.4)	0.20 (0.7)	0.16 (0.8)	0.26 (0.6)	0.88 (0.02)
RGC	*	0.03 (0.9)	0.42 (0.4)	0.12 (0.8)	0.05 (0.9)	0.08 (0.9)	0.03 (0.9)
	**	0.98 (0.001)	0.92 (0.01)	0.84 (0.04)	0.56 (0.3)	0.87 (0.02)	0.85 (0.03)
Viability after regrowth	*	0.86 (0.03)	0.83 (0.04)	0.63 (0.2)	0.26 (0.9)	0.66 (0.2)	0.81 (0.05)
	**	0.74 (0.1)	0.60 (0.2)	0.36 (0.5)	0.02 (0.9)	0.41 (0.4)	0.88 (0.02)

p values are indicated in brackets; n=6; * - prior to cold storage; ** - after cold storage

Table 3.6: Linear regression equations of viability (before and after regrowth and before and after cold storage) and survival, collar diameter and height increment.

Parameter	Linear regression equations	
	Before cold storage	**After cold storage**
Survival	Sur = - 25.1 + 1.86 TTC (r^2 = 0.81) (before regrowth)	Sur = - 19.9 + 2.36 TTC (r^2 = 0.42)
	Sur = - 45 + 1.94 TTC (r^2 = 0.74) (after regrowth)	Sur = - 26.1 + 2.28 TTC (r^2 = 0.54)
Collar diameter	Dia = 2.49 + 0.03 TTC (r^2 = 0.78) (before regrowth)	Dia = 3.28 + 0.03 TTC (r^2 = 0.16)
	Dia = 2.17 + 0.04 TTC (r^2 = 0.69) (after regrowth)	Dia = 2.28 + 0.04 TTC (r^2 = 0.36)
Height increment	Inc = 2.71 + 0.14 TTC (r^2 = 0.66) (before regrowth)	Inc = - 0.4 + 0.26 TTC (r^2 = 0.75)
	Inc = 0.98 + 0.14 TTC (r^2 = 0.64) (after regrowth)	Inc = 0.17 + 0.22 TTC (r^2 = 0.78)

Sur - survival; Dia - collar diameter; Inc - height increment

Although the presence of white roots demonstrates that the roots are not dormant, and this would be expected to be deleterious to the seedling, Johnson-Flanagan and Owens (1985) have found that there is a correlation between number of white roots and RGC. Thus, if the reported relationship between high RGC and high survival following outplanting (Burdett, 1979) is true, which numerous researchers have shown is not (McKay, 1992, van den Drissche, 1992), the present results indicate that the high number of white roots and high RGC on Nov. 14 should result in high survival.

Root class has been suggested to be more important than root biomass in controlling seedling survival (Johnson-Flanagan and Owens, 1985). Work by Stone et al. (1962) demonstrated that the highest survival was associated with high production of absorbing roots. In that case, high RGC was not coincident with absorbing roots. In the present study, Nov. 14 samples not only had high RGC, they had the highest proportion of absorbing roots.

Table 3.7: Correlation coefficients (r) between carbohydrates and field performance.

Parameter		Survival	Collar diameter	Most distal needle length	Height at planting	Total height	Height increment
Reducing carbohydrate	*	0.24 (0.6)	0.52 (0.3)	0.21 (0.7)	- 0.01 (0.9)	0.21 (0.7)	0.32 (0.5)
	**	0.64 (0.2)	0.79 (0.06)	0.73 (0.1)	0.60 (0.2)	0.72 (0.1)	0.41 (0.4)
Total soluble carbohydrate	*	- 0.26 (0.6)	- 0.16 (0.8)	0.48 (0.3)	0.71 (0.1)	- 0.47 (0.3)	- 0.11 (0.8)
	**	0.72 (0.1)	0.81 (0.05)	0.54 (0.3)	0.18 (0.7)	0.56 (0.2)	0.66 (0.2)
Starch	*	0.26 (0.6)	0.18 (0.7)	0.34 (0.5)	0.36 (0.5)	0.32 (0.5)	- 0.09 (0.9)
	**	0.84 (0.04)	0.74 (0.1)	0.81 (0.05)	0.64 (0.2)	0.82 (0.05)	0.51 (0.3)
Total non-structural carbohydrate	*	- 0.09 (0.9)	0.05 (0.9)	- 0.25 (0.6)	0.44 (0.4)	- 0.25 (0.6)	- 0.14 (0.8)
	**	0.83 (0.04)	0.86 (0.03)	0.71 (0.1)	0.37 (0.5)	0.71 (0.1)	0.66 (0.2)

p values are indicated in brackets; n=6; * - prior to cold storage; ** - after cold storage

Comparisons between root growth prior to and after cold storage demonstrated that root growth was lower after storage and was restricted to absorbing roots. These are important in absorption.

RGC was higher on all dates, except Sep. 19 following storage. This suggests that storage had a deleterious effect on the seedlings lifted on Sep. 19, but actually improved the potential for renewed root growth on all other lifting dates. Once again, Nov. 14 seedlings had the highest number of white roots and the highest RGC, further supporting the contention that seedlings lifted on Nov. 14 were superior to the other lifting dates.

Carbohydrate changes have been measured throughout the lifting, storing and thawing period in numerous conifer seedlings (Camm et al., 1995; Cannell et al., 1990; Ritchie, 1982; Ritchie, 1987). Some of these studies have shown very subtle changes in individual carbohydrates (Levesque, 1996). There is little evidence to support the idea that these subtle changes reflect metabolic changes or that they are in any way conferring stress tolerance. Despite this, the trend seems to be towards more complex methodology and expensive equipment, while basic questions remain unanswered. For example, "Which carbohydrates are the best measure of fitness?" and, "Can these be measured in a nursery setting?"

Poor seedling performance after outplanting has been attributed to reduced carbohydrate concentrations in several species, such as Engelmann spruce (*Picea engelmannii*), Sitka spruce and Douglas-fir (Farmer 1978, Ronco, 1973). On the other hand, Deans et al. (1990) did not find any relationship between total non-structural carbohydrate and field performance in Sitka spruce. Recent work by Jiang et al. (1994) showed that total non-structural carbohydrate decreased during cold storage. By comparing fall-lifted, cold-stored seedlings and spring-lifted seedlings, they were able to see that this decrease in total non-structural carbohydrate resulted from decreased starch, which in turn was associated with low initial rates of root growth. This did not, however, translate into lower growth or survival at the end of the growing season.

The objective of the present study was to determine whether measurements of reducing, total soluble, total non-structural carbohydrates, or starch would provide fast and

accurate methods to assess seedling stock for subsequent outplanting success and seedling productivity. Carbohydrates as potential indicators of a seedling's condition have several important practical advantages over the RGC test and other tests that are currently used to assess stock quality. Starch analysis, together with total soluble carbohydrate analysis and total non-structural carbohydrate determination, can be completed in 2 days, requires relatively inexpensive equipment (a spectrophotometer of any type), within-batch variation is low, and since only small quantities of root material are needed (approximately 300 mg fresh weight) the analyses are essentially nondestructive.

In order to study seedlings with a broad range of carbohydrate contents, seedlings were lifted to cold storage before the onset of chilling, during chilling, soon after the chilling requirement had been met and after the dormancy requirement had been met. The results from the present study show that starch and total non-structural carbohydrate, assessed after storage, are good indicators of survival. RGC was a good indicator of survival and seedling performance in the first season following outplanting. On the other hand, carbohydrate levels did not provide the best indication of subsequent seedling performance. While collar diameter was significantly correlated with carbohydrates, height increment was not.

Although no one class of carbohydrate was correlated significantly (at the 95% confidence level) with survival, collar diameter and height increment, there are indications that they may be useful predictors. Both total soluble carbohydrate and total non-structural carbohydrate were significantly correlated with survival and field performance at the 80% confidence level. (At this level, reducing carbohydrate was significantly correlated with survival and collar diameter). Further, total soluble carbohydrate and total non-structural carbohydrate were significantly correlated with RGC, which in turn, was a good measure of survival and field performance.

The results from the present study show that TTC reduction as a measure of root viability when measured prior to storage is the best predictor of both survival and field performance. Significant correlations were found between viability and each of survival, collar diameter and height increment. The highest correlations were for data collected before cold storage and in the absence of regrowth. These correlations have not been demonstrated

before and indicate that TTC reduction has good potential as a pre-storage predictor of survival and field performance following outplanting.

TTC reduction measures respiratory activity (Joslin and Henderson, 1984). Thus, we can see that the roots were relatively inactive on Sep. 19. As respiration is a very good measure of metabolic activity, these results point towards a serious problem in the seedlings lifted on Sep. 19, which was not rectified by cold storage. Interestingly, high respiratory activity was measured on Nov. 14, the lifting date that performed the best in the field.

The results from the present study demonstrate the utility of the static model for chilling (Hänninen, 1990), but also demonstrate that lifting should not be postponed once the chilling requirement has been met. According to the static model for chilling hours in 1995 (Appendix V), the seedlings began to accumulate chilling hours on Sep. 28 and the requirement for storage (500 chilling hours) was met by Nov. 5 (Rob Scagel, pers. comm.). Dormancy requirements were satisfied by Nov. 29. Although the seedlings would be expected to maintain their metabolic and developmental status for a number of months, warmer temperatures at the end of Nov. 1995 obviously led to changes in the root system (Appendix VI). This may result from the fact that the root system as a whole is rarely, if ever dormant. Root growth is episodic, with some roots growing while others are dormant or quiescent (Johnson-Flanagan and Owens, 1985). This is demonstrated in RGC tests. In studies of white spruce (Johnson-Flanagan and Owens, 1985) and in the present study of lodgepole pine, there was significant RGC throughout the study period. In fact, the highest RGC values were recorded on Nov. 14, when seedlings would be predicted to be dormant.

Seedlings continue to respire at low rates while in storage, slowly utilizing the available carbohydrate reserves (van den Driessche, 1979). In the present study, total non-structural carbohydrate decreased in response to cold storage. The same trend was found for white spruce (Jiang et al., 1994), Douglas-fir (Ritchie, 1982), and Sitka spruce (Cannell et al., 1990). These researchers concluded that the loss of total non-structural carbohydrate resulted mainly from a decline in soluble carbohydrate rather than starch. In contrast, the decrease in total non-structural carbohydrate in the present study was mostly a result of starch decline.

Similarly, Chomba et al. (1993) found that Engelmann spruce root soluble carbohydrate was not strongly affected by four months of cold storage, but that starch content was drastically decreased by cold storage over the study period. It is not clear why different sources of carbohydrates are utilized by different species.

The physiological state of the seedlings during storage appears to be more important than time in cold storage. Seedlings lifted in December lost more total non-structural carbohydrate than did those lifted in November. This higher rate of depletion may relate to increased metabolism associated with quiescence , as the dormancy requirement was met on Nov. 29. This could also explain the apparent temporal response up to November as seedlings were accumulating chilling hours up to that point.

In the present study, the increased height at planting measured for lifting dates from September to December was not beneficial for subsequent growth of the seedlings during the first field season. To my knowledge, this has not been reported previously. Seedlings lifted on Nov. 14 showed the highest increment in 1996 even though they were smaller at planting time than were seedlings lifted in December.

In conclusion, the results from the present study indicate that root viability prior to cold storage, measured as TTC reduction, may be a very good predictor of seedling survival and field performance following outplanting. It is a rapid, inexpensive and simple method, which is essentially non-destructive. It can be used before cold storage and it is a great improvement over the RGC test, which must be done after cold storage in order to be accurate. Finally, it appears that TTC reduction may be useful as a general predictor, as the regression equations were, in general, similar under four different conditions. There is also potential to use carbohydrates as predictors. However, in this case, additional work is required to determine if the level of significance is acceptable.

3.5. Literature Cited

Bigras, F.J., and D'Aoust, A.L. 1993. Influence of photoperid on shoot and root frost tolerance and bud phenology of white spruce seedlings (*Picea glauca*). Can. J. For. Res. 23:219-228.

Burdett, A.N. 1979. New methods for measuring root growth capacity: their value in assessing lodgepole pine stock quality. Can. J. For. Res. 9:63-67.

Burdett, A.N., and Simpson, D.G. 1984. Lifting, grading, packaging. *In* Forestry nursery manual: production of bareroot seedlings. Edited by M.L.Duryea and T.D.Landis. Forest Research Laboratory, Oregon State University, Corvallis, Nijhof/Junk, The Hague, Boston, pp. 227-234.

Camm, E.L., and Harper, G.J. 1991. Temporal variations in cold sensitivity of root growth in cold-stored white spruce seedlings. Tree Physiol. 2:425-431.

Camm, E.L., Guy, R.D., Kubien, D.S., Goetze, D.C., Silim, S.N., and Burton, P.J. 1995. Physiological recovery of freezer-stored white and Engelmann spruce seedlings planted following different thawing regimes. New For. 10:55-77.

Cannell, M.G.R., Tabbush, P.M., Deans, J.D., Hollingsworth, M.K., Sheppard, L.J., Philipson, J.J., and Murray, M.B. 1990. Sitka spruce and Douglas-fir seedlings in the nursery and in cold storage: root growth potential, carbohydrate content, dormancy, frost hardiness and mitotic index. Forestry. 63:9-27.

Chomba, M.B., Guy, R.D., and Weger, H. 1993. Carbohydrate reserves accumulation and depletion in Engelmann spruce (*Picea engelmannii*, Parry): effect of storage and pre-storage CO_2 enrichment. Tree Physiol. 13:351-364.

Deans, J.D., Lundberg, C., Tabbush, P.M., Cannell, M.G.R., Sheppard, L.J., and Murray, M.B. 1990. The influence of desiccation, rough handling and cold storage on the quality and establishment of Sitka spruce planting stock. Forestry. 63:129-141.

Dubois, M., Gilles, K.A., and Hamilton, J.K. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28:350-360.

Farmer, R.E.Jr. 1978. Seasonal carbohydrate levels in roots of Appalachian hardwood planting stock. Tree Plant. Notes 29(3):22-24.

Hänninen, H. 1990. Modeling bud dormancy release in tree from cool and temperate regions. Acta For. Fenn.213:47-53.

Jiang, Y., Zwaizek, J.J., and Macdonald, S.E. 1994. Effects of prolonged cold storage on carbohydrate and protein content and field performance of white spruce bareroot seedlings. Can. J. For. Res. 24:1369-1375.

Johnson-Flanagan, A.M., and Owens, J. N. 1985. Root growth and root growth capacity of white spruce (*Picea glauca* [Moench] Voss) seedlings. Can. J. For. Res. 15:625-630.

Joslin, J.D., and Henderson, G.S. 1984. The determination of percentages of living tissue in woody fine root samples using triphenyltetrazolium chloride. For. Sci. 30:965-970.

Levesque, F. 1996. Carbohydrates in white spruce and lodgepole pine seedlings during winter, outdoors, in freezer storage and thawing. M.Sc.Thesis, University of British Columbia. Vancouver, 131 pp.

Lindström, A., and Nyström, C. 1987. Seasonal variation in root hardiness of container-grown Scots pine, Norway spruce, and lodgepole pine seedlings. Can. J. For. Res. 17:787-793.

McKay, H.M. 1992. Electrolyte leakage from fine roots of conifer seedlings: a rapid index of plant viability following cold storage. Can. J. For. Res. 22:1371-1377.

McKay, H.M., and Mason, W.L. 1991. Physiological indicators of tolerance to cold storage in Sitka spuce and Douglas-fir seedlings. Can. J. For. Res. 21:890-901.

Ritchie, G.A. 1982. Carbohydrate concentration reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Can. J. For. Res. 12:905-912.

Ritchie, G.A. 1987. Some effects of cold storage on seedling physiology. Tree Plant. Notes, 38:11-15.

Ritchie, G.A., and Dunlop, J.R. 1980. Root growth potential: its development and expression in forest tree seedlings. N.Z.J. For. Sci. 10:218-248.

Ronco, F. 1973. Food reserves of Engelmann spruce planting stock. For. Sci. 19:213-219.

Ross, A.F. 1959. Part II. Dinitrophenol method for reducing sugars, In Potato Processing, Ed. by W.F. Talburt, and O. Smith. The AVI Publishing Company Inc. Westport Connecticut. pp. 469-470.

Rose, R., C.L., Rose, S.K., Omi, K.R., Forry, D.M., Durall, and Bigg, W.L. 1991. Starch determination by perchloric acid vs enzymes: Evaluating the accuracy and precision of six colorimetric methods. J. Agric. Food Chem. 39:2-11.

Sharpe, A.L., and Mason, W.L. 1992. Some methods of cold storage can seriously affect root growth potential and root moisture content and subsequent forest performance of Sitka spruce and Douglas fir transplants. Forestry, 65:463-476.

Steponkus, P.L. and Lanphear, F.O. 1967. Refinement of the triphenyl tetrazolium chloride method of determining cold injury. Plant Physiol. 42:1423-142.

Stone, E.C., Jenkinson, J.L., and Krugman, S.L. 1962. Root-regenerating potential of Douglas-fir seedlings lifted at different times of the year. For. Sci. 8:288-297.

van den Driessche, R. 1979. Respiration rate of cold-stored nursery stock. Can. J. For. Res. 9:15-18.

van den Driessche, R. 1992. Changes in drought resistance and root growth capacity of container seedlings in response to nursery drought, nitrogen, and potassium treatments. Can. J. For. Res. 22:740-749.

Vidaver W., Binder W., Brooke R.C., Lister G.R., and Toivonen, P.M.A. 1989. Assessment of photosynthetic activity of nursery grown *Picea glauca* seedlings using an integrating fluorometer to monitor variable chlorophyll fluorescence. Can. J. For. Sci. 19:1478-1482.

Warrington, I.J., and Rook, D.A. 1980. Evaluation of the techniqes use in determining frost tolerance of forest planting stock: A review. N.Z.J. For. Res. 10;116-132.

CHAPTER IV

EFFECTS OF SLOW AND FAST COOLING ON ROOT GROWTH AND MORPHOLOGY, AND ROOT AND NEEDLE CARBOHYDRATE RESERVES IN LODGEPOLE PINE SEEDLINGS

4.1 Introduction

Foresters generally consider cold storage to be a benign method for maintaining seedlings until designated plantation areas are free of snow. This, unfortunately, is not the case. Numerous studies have demonstrated the deleterious effects of prolonged storage (Camm and Harper, 1991; Cannell et al., 1990; Jiang et al., 1995; Puttonen, 1986; Ritchie, 1982; Venn, 1980). Tissues are known to remain metabolically active at temperatures above, and even slightly below freezing (Marshall and Waring, 1985). Troeng (1991) reported that respiration continues in cold-stored seedlings. Such respiration depletes seedling carbohydrate reserves (Ritchie, 1982). Studies on Douglas-fir and Sitka spruce (Cannell et al., 1990), radiata pine (McCraken, 1979), ponderosa pine (Omi et al., 1994), Engelmannn spruce (Ronco, 1972), and white spruce (Jiang et al., 1994) all showed losses of starch during cold storage. In fact, Forry and Zaerr (1988) reported that root starch reserves were completely utilized during cold storage of Douglas-fir. The effects of storage are also manifested as losses in chlorophyll (Camm et al., 1993, Mattsson and Troeng, 1986), decreases in RGC (Burr et al., 1988; Carlson, 1985; Tabbush, 1988) and decreases in survival (Stone and Jenkinson, 1970). The effects of storing seedlings that have not acquired sufficient chilling hours has also been demonstrated. In addition, the effects of different thawing rates such as slow (thawing for 9 days at 5-15°C) and rapid (3-4 hours in dark at 21°C) have been studied (Camm et al., 1995; Levesque, 1996). What has not been addressed is the effect of cooling rate on seedling health.

Currently, seedlings are cooled in boxes of 250 seedlings. This means that the seedlings on the inside of box cool at a much slower rate than do the seedlings on the outside of the box. The objective of this study was to assess the effect of slow (center region of the

box) and fast (outermost region in the box) cooling rates on seedling health. The effect of storage time on root carbohydrates was also examined. In addition, this study re-examines the concentrations of different types of carbohydrates in relation to one another and RGC, in order to gain further insight into the possible correlations reported in Chapter III. This portion of the study is ongoing and will include field results when complete.

4.2. Material and Methods

4.2.1 Plant Material

Lodgepole pine (*Pinus contorta* Dougl. spp. *latifolia*) seedlings (seedlot #30862: 1500 m elevation; 50°54'N; 121°08'W) were grown as previously described (Chapter 3.2.1) lifted from the greenhouse (Pitt Meadows, British Columbia) on September 23, November 14, and December 06, 1996.

4.2.2 Pre-storage Sampling

Of the 420 seedlings lifted on each date (September 23, November 14, December 06, 1996), 30 were used to determine the total number of elongating, absorbing and brown roots on the whole root system. All roots studied were long laterals and were classified as elongating (white and lacking of root hair), absorbing (hair-covered white and possessing root hairs), and brown (the root being enveloped by a necrotic layer). After counting the number of roots on the whole root system, the seedlings were analyzed for reducing carbohydrate, total soluble carbohydrate and starch content in both needle and roots as described in Chapter III. Roots were washed in cold tap water and the central root mass of each seedling was sampled by removing a 2 cm wide band of roots at a distance of approximately 1 cm from the bottom of the root plug. Total non-structural carbohydrate was calculated as described in Chapter III (3.26.4). Chlorophyll a and b and total chlorophyll were determined in the needles. Needles were taken from 20 mm below the bud. A sample of an additional 10 seedlings was used to determine RGC.

4.2.3 Post-storage Experiment

Seedlings were stored at - 2°C in the dark (3-4.5 months). Seedlings were shipped (refrigerated) overnight courtesy of Mike Susak from Rutland Cold storage, Hawkeye Holdings Ltd., Kelowna, B.C. Seedlings entering cold storage were cooled at two different rates (Fig. 4.1).

Fast cooling/slow thawing

Twelve bundles (10 seedlings each) with cellophane wrapped root plugs were placed inside a waxed box containing a plastic-lined paper bag (the bundles did not touch each other; there was space between them), and stored in cold storage at - 2°C. The temperature

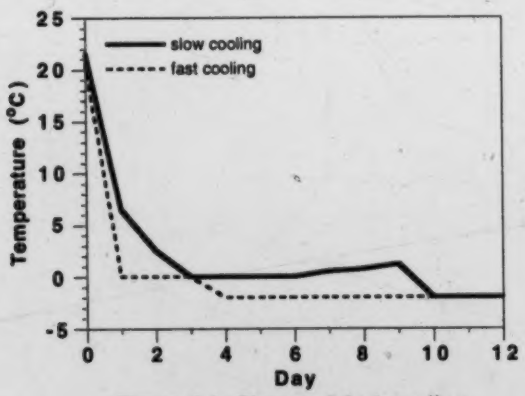


Figure 4.1: Slow and fast cooling

inside the center of the bundle was monitored daily with a probe thermometer (± 0.5°C) until the temperature reached - 2°C. This took 4 days (Fig. 4.1). The cold storage temperature was constantly monitored and remained at - 2°C throughout the storage period. The lifting and assessment dates are shown in Table 4.1. After 1 month of cold storage, 2 bundles were taken out of cold storage and thawed at + 4°C in a cold room for 7 days. Five seedlings were taken from the center of each bundle and subjected to root total soluble carbohydrate determination. The same procedure was repeated for each of the lifting dates, one month prior to thawing. At the end of cold storage, boxes of seedlings were moved to 4°C to thaw for 7 days. From the 10 remaining bundles, the 4 central seedlings were taken to make a

sample of 40 seedlings. The seedlings were subjected to determination of the same parameters outlined above.

Slow cooling/slow thawing

Twenty-six bundles (10 seedlings each) were prepared as above and placed in cold storage at - 2°C. In this case, the bundles were compactly packed. After 10 days, the temperature inside the bundle from the center of box reached - 2°C (Fig. 4.1).

Table 4.1: Lifting and outplanting dates in 1996.

Lifting Dates	September 23	November 14	December 6
Prior to cold storage assessment date	September 23, 1996	November 14, 1996	December 6, 1996
Days between prior to cold storage and first assessment date	30	30	30
First assessment date	October 23, 1996	December 14, 1996	January 6, 1997
Days between first and second assessment date	98	64	69
Second assessment date	January 28, 1997	February 15, 1997	March 15,1997
Days between second and after cold storage assessment date	32	29	31
After cold storage assessment date	February 28, 1997	March 15, 1997	April 16, 1997
Days between after cold storage assessment and outplanting dates	96	81	49
Outplanting dates	June 4, 1997	June 4, 1997	June 4, 1997

Seedlings were sampled as outlined in Table 4.1 with slow thawing as described above. At the end of cold storage, boxes of seedlings were moved to 4°C to thaw for 7 days. Ten bundles (from 26 bundles) from the center of the box were removed and the 4 central

seedlings from each bundle were pooled to make a 40 seedling sample. The seedlings were subjected to the same assessments as above.

4.2.4 Sampling for Root Carbohydrates and Starch

Sampling was done as described in Chapter III.

4.2.5 Needle Reducing Carbohydrate and Total Soluble Carbohydrate Content

Needle samples (0.05 g of fresh weight) were taken 20 mm below the apical bud, ground into a fine powder in liquid nitrogen with a mortar and pestle, and extracted in 3.0 mL phosphate buffer (20 mM, pH 7.2). The crude homogenate was centrifuged at 2,200 rpm for 10 minutes. The supernatant was analyzed reducing and total soluble carbohydrates as previously described (Chapter III, 3.2.6.1; 3.2.6.2).

4.2.6 Needle Starch Content

Needle tissue (0.25 g fresh weight) was taken 20 mm below the apical bud, ground into a fine powder in liquid nitrogen with a mortar and pestle, and extracted three times in 5.0 mL of methanol:chloroform:water (MCW) (12:5:3 v/v/v), vortexed for 5 minutes, centrifuged at 2,200 rpm for 10 minutes and the supernatant carefully aspirated. The remaining MCW was evaporated to dryness by placing the rack of open tubes in an oven at 50°C for 4 h. The pellet was subjected to the same procedure for starch determination as previously described (Chapter III, 3.2.6.3).

4.2.7. Total Chlorophyll, and Chlorophyll a and b Content

Chlorophylls were measured in needle samples (0.2 g) taken 20 mm below the apical bud as follows: individual samples were ground in liquid nitrogen into a fine powder and then 1 mL of 80% acetone was added while grinding continued. The slurry was transferred to 2 mL screwcap tubes. The mortar and pestle were rinsed with 1 mL of 80% acetone. This was added to the microfuge tube. The sample was microcentrifuged at 13,000 rpm at 4°C for 10 minutes. The supernatant was transferred to a flask and kept on ice in the dark. The pellet was resuspended in 300 µL 80% acetone. After centrifugation, as above, the supernatants were

combined. The pellet was extracted in 80% acetone, as above, until the pellet was white (approximately 6 times). The supernatant was transferred to a 10 mL volumetric flask and the volume was adjusted with 80% acetone. Absorbance was read at 665 nm and 649 nm. The chlorophyll content in the sample was calculated by using the following equations (Vernon, 1960):

$$\text{chlorophyll a (mg/L)} = 11.63\ (A_{665}) - 2.39\ (A_{649}) \qquad \textit{(Equation 4.1)}$$

$$\text{chlorophyll b (mg/L)} = 20.11\ (A_{649}) - 5.18(A_{665}) \qquad \textit{(Equation 4.2)}$$

$$\text{total chlorophyll (mg/L)} = 6.45\ (A_{665}) + 17.72(A_{649}) \qquad \textit{(Equation 4.3)}$$

4.2.8 Outplanting

The seedlings (80 per lifting date, 40 slow and 40 fast cooling) were removed from cold storage and thawed for 7 days at + 4°C according to the schedule outlined in Table 4.1. The seedlings were planted on a clear-cut site near Kelowna, British Columbia. The site was provided courtesy of Kelly Fay from Riverside Forest Products, Kelowna, BC.

4.2.9 Statistical Analysis

Differences in number of elongating roots, percentage of white roots, RGC, reducing carbohydrates (root and needle), total soluble carbohydrate (needle), starch (root), total non-structural carbohydrate (root), and chlorophyll prior to and after cold storage (slow and fast cooling) at each lifting date were determined with sources of variations of least difference (LD) and experimental error using one-way ANOVA. Differences in the same parameters were determined between lifting dates for prior to and after cold storage (slow and fast cooling) using separate one-way ANOVA's. Differences in total soluble carbohydrate prior to storage, during storage and after cold storage were determined using one-way ANOVA. Specific differences among LD means were determined with the least significant difference (LSD) test. All data parameters listed above were analyzed by two-way ANOVA (lifting dates versus treatments - prior to and after cold storage, slow and fast cooling). A significance level of $p<0.05$ was used. All statistical calculations were performed using the General Linear Model of SAS 6.10. program (SAS Institute Inc., Carry, NC 27513, USA).

4.3 Results

Three sampling dates were selected for the study in 1996. Seedlings had not been exposed to chilling by the September date. By November 14 the chilling requirement had been met and by December 6 the dormancy requirement had been met (Appendix VII).

The number of roots in each class, elongating, absorbing and brown, were monitored in the root system for each lifting date (prior to cold storage). There were no differences in the number of absorbing and brown roots between lifting dates, but seedlings lifted on Dec. 6, had fewer elongating roots than those lifted on Nov. 14. (Fig. 4.2a).

The effect of cold storage and cooling rates can be seen in Figure 4.2b. On all dates, the root system had a significantly higher (LSD, $p<0.05$) percentage of white roots prior to cold storage than after cold storage, for both slow and fast cooling. As noted in the 1995 study, seedlings lifted in mid-November had significantly more (LSD, $p<0.05$) white roots prior to cold storage relative to the other lifting dates (Chapter III; Figure 3.1b). Comparisons between slow and fast cooling showed that slow cooling resulted in a significantly higher (LSD, $p<0.05$) percentage of white roots for Nov. 14 and Dec. 6 lifting dates. There was no significant interaction between percent of white roots prior to and after cold storage for both slow and fast cooling rates and lifting dates ($p>0.2$). Following cold storage there were no elongating roots, few absorbing roots and a high number of brown roots for both fast and slow cooling (Fig. 4.3a).

RGC prior to and after cold storage was significantly lower (LSD, $p<0.05$) in September than on the other two dates (Fig. 4.3b). The response to cold storage and cooling rates was dependent upon the lifting dates. There was a significant interaction between RGC prior to and after cold storage for both slow and fast cooling rates and lifting dates ($p<0.001$). Slow cooling resulted in significantly higher (LSD, $p<0.05$) RGC values relative to fast cooling for the Nov. 14 and Dec. 6 lifting dates.

The distribution of white roots in the root plug following prior to cold storage regrowth is presented on Fig. 4.4a,b. White roots were predominantly located at the bottom of the root plug under all conditions. Fast cooling led to a significantly higher (LSD, $p<0.05$) percentage of white roots in the bottom of the plug on all lifting dates (Fig. 4.4b).

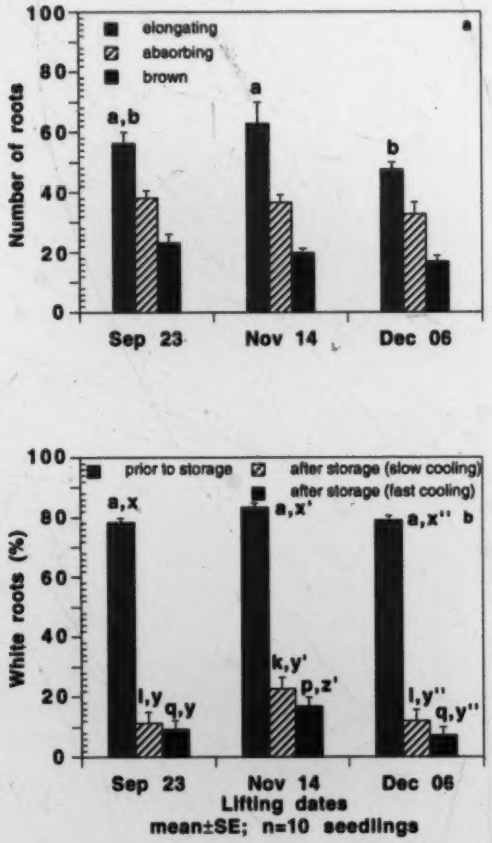


Figure 4.2: Root morphology data for lodgepole pine seedling prior to and after cold storage

a) Number of elongating, absorbing and brown roots prior to cold storage. Means having common letter(s) are not significantly different ($p>0.05$) prior to cold storage (a, b, c) for elongating roots according to a one-way ANOVA and LSD test.

b) Percent white roots (sum of elongating and absorbing). Means having common letter(s) are not significantly different ($p>0.05$) prior to cold storage (a, b, c), after cold storage (slow cooling; k, l, m), and after cold storage (fast cooling; p, q, r) according to a one-way ANOVA and LSD test.

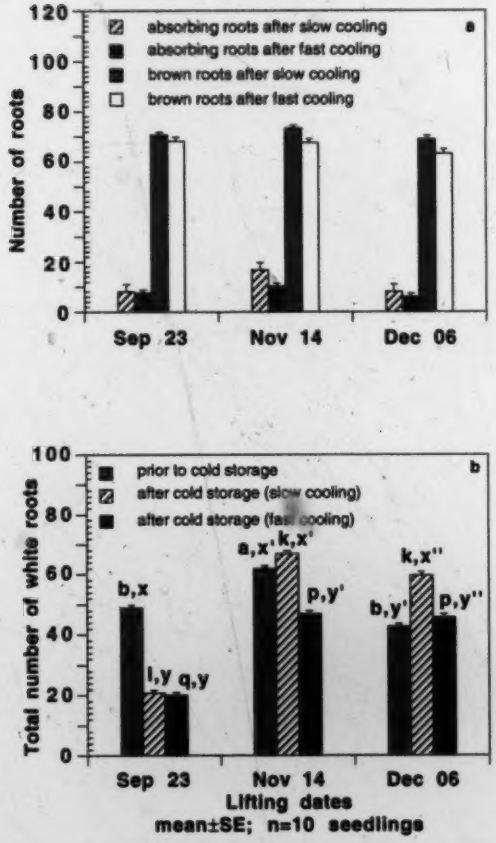


Figure 4.3: Root morphology data and RGC for lodgepole pine seedlings prior to and after cold storage

a) Number of absorbing and brown roots after cold storage. Data were not analyzed by ANOVA.
b) RGC prior to and after cold storage. Means having common letter(s) are not significantly different ($p>0.05$) prior to cold storage (a, b, c), after cold storage (slow cooling; k, l, m), and after cold storage (fast cooling; p, q, r) according to a one-way ANOVA and LSD test. Means having common letters (x, y, z) are not significantly different ($p>0.05$) for a lifting date according to a one-way ANOVA and LSD test.

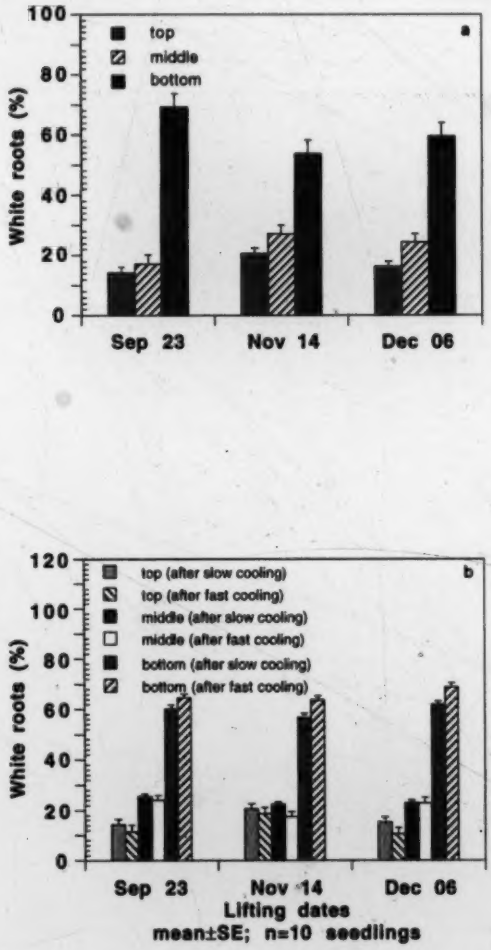


Figure 4.4: Root morphology following RGC for lodgepole pine seedlings prior to and after cold storage

a) Percent white roots in different regions of the root system prior to cold storage. Data were not analyzed by ANOVA.
b) Percent white roots in different regions of the root system after cold storage (slow or fast cooling). Data were not analyzed by ANOVA.

Prior to cold storage, the amount of reducing carbohydrate in the root was significantly higher (LSD, $p<0.05$) on Nov. 14, than on the other two dates (Fig. 4.5a). This advantage was lost following storage. The response to cooling rate was variable but a significant interaction occurred between reducing carbohydrate prior to and after cold storage for both slow and fast cooling rates and lifting date ($p<0.0001$).

The changes in root total soluble carbohydrate during storage were examined by sampling prior to cold storage, twice during cold storage (first and second assessment) and after cold storage (Table 4.2). Total soluble carbohydrate content prior to cold storage was significantly higher on Nov. 14 (LSD, $p<0.05$) as compared with the other dates. Cold storage initially led to an increase in total soluble carbohydrate. The greatest increase was in response to slow cooling in the December samples. Fast cooling consistently resulted in lower total soluble carbohydrate during storage relative to slow cooling.

Table 4.2: Fluctuations in total soluble carbohydrate during cold storage.

Stage in cold storage	Lifting Dates September 23	November 14	December 6
Prior to cold storage	7.3±0.74^{c,d}	9.2±1.07^{b}	8.1±0.64^{d}
First assessment (a month in cold storage)			
slow cooling	10.2±0.46^{a}	18.1±1.32^{a}	22.3±0.90^{a}
fast cooling	8.9±0.44^{a,b}	16.6±0.99^{a}	15.2±0.63^{b}
Second assessment (a month prior to thawing)			
slow cooling	6.5±0.26^{d,e}	9.3±0.79^{b}	14.8±0.59^{b,c}
fast cooling	1.9±0.14^{f}	8.8±0.59^{b}	11.9±0.89^{c}
After cold storage			
slow cooling	8.46±0.21^{b,c}	9.5±0.46^{b}	4.3±0.68^{e}
fast cooling	5.4±0.61^{e}	8.8±0.31^{b}	3.4±0.48^{e}

mean±SE; means within columns having common letter(s) are not significantly different at $p>0.05$;

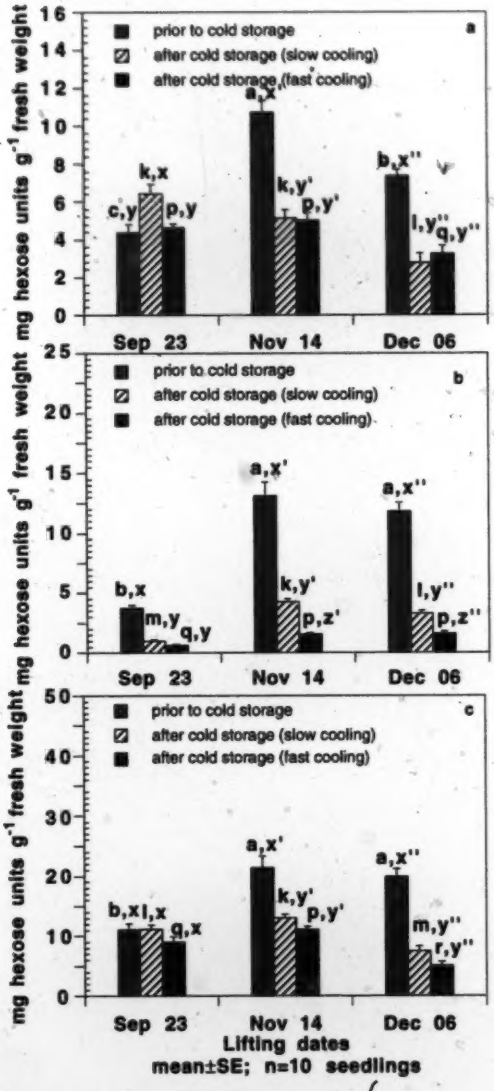


Figure 4.5: Carbohydrate content in lodgepole pine seedling roots

a) Reducing carbohydrate content prior to and after cold storage (slow and fast cooling). Means having common letter(s)are not significantly different ($p>0.05$) prior to cold storage (a, b, c), after cold storage (slow cooling; k, l, m), and after cold storage (fast cooling; p, q, r) according to a one-way ANOVA and LSD test. Means having common letter(s) (x, y, z) are not significantly different ($p>0.05$) at a lifting date according to a one-way ANOVA and LSD test.

b) Starch content prior to and after cold storage (slow and fast cooling). Statistics are as described for Fig. 4.5a.

c) Total non-structural carbohydrate content prior to and after cold storage (slow and cooling). Statistics are as described for Fig. 4.5a.

The results for root starch content are shown in figure. 4.5b. Seedlings lifted in November and December had significantly higher (LSD, $p<0.05$) amounts of starch than those from September, prior to cold storage; cold storage decreased starch content; and the effect was significantly greater (LSD, $p<0.05$) in response to fast cooling for seedlings lifted in November and December. There was a significant interaction between starch content prior to and after cold storage for both slow and fast cooling and lifting date ($p<0.0001$).

Total non-structural carbohydrate was assessed as the sum of total soluble carbohydrate and starch (Fig. 4.5c). Relative to slow cooling, fast cooling led to significantly greater (LSD, $p<0.05$) decreases in total non-structural carbohydrate after cold storage in seedlings lifted on Nov. 14 and Dec. 6. The seedlings lifted on Dec. 6 showed the largest decrease in total non-structural carbohydrate after cold storage relative to seedlings lifted on the other two dates. There was a significant interaction between total non-structural carbohydrate content prior to and after cold storage for both slow and fast cooling and lifting date ($p<0.0001$).

Examination of the reducing carbohydrate content in needles showed little change in response to cold storage (Fig. 4.6a). Values were significantly different (LSD, $p<0.05$) between lifting dates prior to cold storage, with the September samples having the least reducing carbohydrate. No significant interactions occurred between needle reducing carbohydrate prior to and after cold storage for both slow and fast cooling and lifting date ($p>0.6$). The total soluble carbohydrate content was also significantly lower (LSD, $p<0.05$) in September relative to the other two lifting dates, prior to cold storage (Fig. 4.6b). Unlike reducing carbohydrate, there was a significant response to cold storage and cooling rate. Slow cooling increased total soluble carbohydrate content relative to contents prior to cold storage in the September and November samples. Fast cooling caused a decrease in samples lifted in November and December and an increase in the September samples relative to measurements prior to cold storage. There was a significant interaction between total soluble carbohydrate content prior to and after cold storage for both slow and fast cooling and lifting date ($p<0.001$). Starch content in the needles was below the detection limits of the method, thus neither starch nor total non-structural carbohydrates are reported.

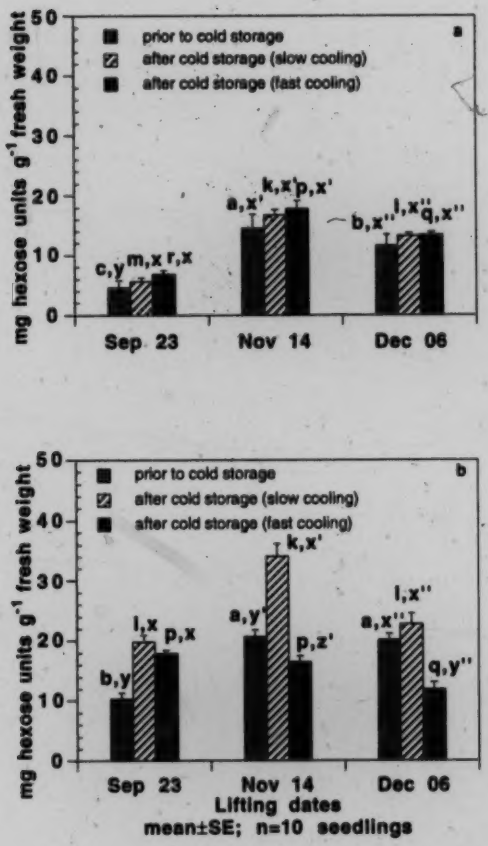


Figure 4.6: Carbohydrate content in needles of lodgepole pine seedlings

a) Reducing carbohydrate content prior to and after cold storage (slow and fast cooling). Means having common letter(s) are not significantly different ($p>0.05$) prior to cold storage (a, b, c), after cold storage (slow cooling; k, l, m), and after cold storage (fast cooling; p, q, r) according to a one-way ANOVA and LSD test. Means having common letter(s) (x, y, z) are not significantly different ($p>0.05$) at a lifting date according to a one-way ANOVA and LSD test.

b) Total soluble carbohydrate content prior to and after cold storage (slow and fast cooling). Statistics are as described for Fig. 4.6a.

Chlorophyll contents and chlorophyll a/b ratios were examined in needles (Fig. 4.7a,b; Fig. 4.8a,b). Small changes in chlorophyll a and b content are on Fig. 4.7a,b for seedlings lifted on Sep. 23 and Dec. 6 only.

4.4. Discussion

One of the objectives of the present study was to confirm the relationships noted in 1995. These included the effects of cold storage on seedlings lifted at three different physiological states, the relationship between carbohydrates and RGC (reported in Chapter III), the utility of RGC and carbohydrate analyses as predictors of seedling survival and field performance, and the effect of cold storage on drought stress tolerance (reported in Chapter V).

Differences in seedling physiology were needed to further study these relationships. For this reason, seedlings were lifted prior to chilling (September), once the chilling requirement had been met (November) and after the dormancy requirement had been met (December). Seedling health, measured as RGC and content of reducing carbohydrate, total soluble carbohydrate, starch and total non-structural carbohydrate, before and after cold storage, was again compromised by early lifting. Delaying lifting until after the dormancy requirement had been met also led to decreased seedling health, although the response was less than that measured in 1995. These results indicate that the data set should provide further insight into the relationships noted above. As such, the data have been analyzed and integrated into the appropriate chapters, with the exception of the work requiring field analysis.

The other objectives of the present study were to begin to examine the effect of cooling rates on seedling health, measured as RGC, root and needle carbohydrate and needle chlorophyll content, before and after cold storage and the effect of storage time on root carbohydrates. The results will be compared with field data collected in the fall of 1997. As such, the work reported herein is preliminary in nature and, therefore, is not a comprehensive study.

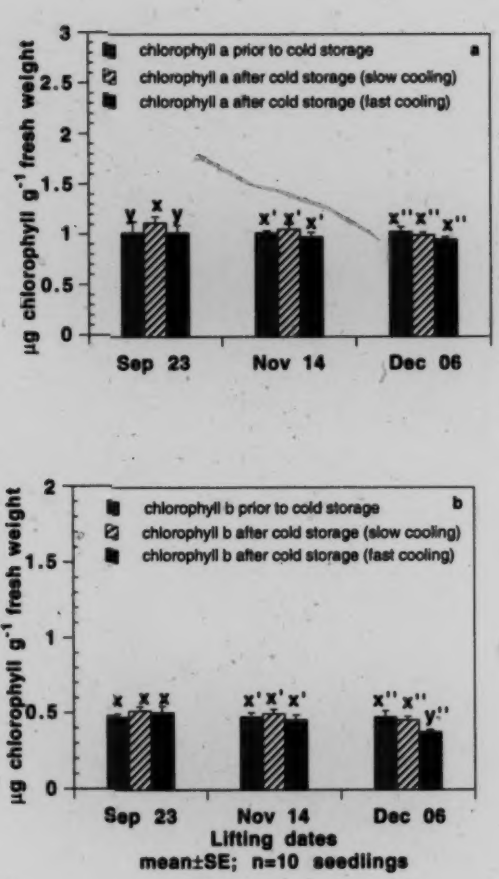


Figure 4.7: Chlorophyll a and b content in needles of lodgepole pine seedlings

a) Chlorophyll a content prior to and after cold storage (slow and fast cooling). Means having common letter(s) (x, y, z) are not significantly different ($p>0.05$) at a lifting date according to a one-way ANOVA and LSD test.
b) Chlorophyll b content prior to and after cold storage (slow and fast cooling). Statistics are as described for Fig. 4.7a.

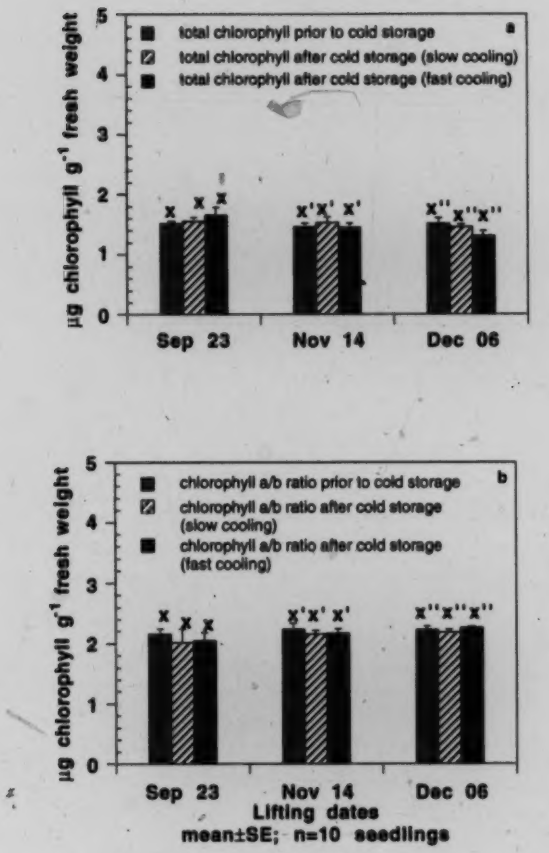


Figure 4.8: Total chlorophyll and chlorophyll a/b ratio in needles of lodgepole pine seedlings

a) Total chlorophyll content prior to and after cold storage (slow and fast cooling). Means having common letter(s) (x, y, z) are not significantly different ($p>0.05$) at a lifting date according to a one-way ANOVA and LSD test.

b) Chlorophyll a/b ratio prior to and after cold storage (slow and fast cooling). Statistics are as described for Fig. 4.8a.

The results from the present study clearly show that slow cooling is superior to fast cooling. RGC, root total soluble carbohydrate, starch and total non-structural carbohydrate, and needle total soluble carbohydrates were all higher following slow cooling. Of these, RGC, root total soluble carbohydrate and total non-structural carbohydrate have been shown to be significantly correlated with outplanting success and subsequent field survival (Chapter III). This result is counterintuitive. Metabolic processes, such as respiration, are temperature dependent (Ritchie, 1982). Ritchie (1987) found that seedlings stored at - 2°C had approximately 2.5 mg more root total non-structural carbohydrates than ones stored at 2°C. Camm et al. (1995) noted that slow thawing promoted respiration and loss of carbohydrate reserves in the root.

Although fast cooling was expected to result in better seedling health, the results of the present study clearly showed that slow cooling is superior to fast cooling in this regard. This may be a reflection of the ability of seedlings to adapt gradually in the case of slow cooling while less adaptation is possible under fast cooling conditions. Further, the slow cooled seedlings were only exposed to temperature above - 2°C for 6 days longer than fast cooled seedlings, so depletion of carbohydrate may not be as great as those reported by Ritchie (1987).

Needle chlorophyll content has been used as a measure of seedling health. In the present study, seedlings underwent significant changes in RGC and carbohydrate content in the absence of changes in chlorophyll content. This suggests that chlorophyll content, while being easy, fast and inexpensive to measure, is not an accurate index of seedling health.

Time in cold storage is expected to be less important in determining seedling health than is rate of thawing (Levesque, 1996). Results from the present study do not support this supposition, as there were significant changes in total soluble carbohydrate during the storage period. In all samples, total soluble carbohydrate increased after one month in cold storage and decreased thereafter. Jiang et al. (1994) also noted changes in carbohydrates during storage of white spruce but in their study, total soluble carbohydrate decreased over the first month while starch increased. Thus, time in storage is obviously an important consideration.

Different findings in physiological changes caused by cold storage can be considered species specific for spruce and pine.

Currently, the industry slow cools nursery stock entering cold storage. Seedlings are boxed and the boxes are packed into cold storage facilities. On the basis of the findings from the preliminary study, it would not be suggested that they alter the slow cooling practice.

4.5 Literature Cited

Burr, K.E., Tinus, R.W., Wallner, S.J., and King, R.M. 1989. Relationships among cold hardiness, root growth potential and bud dormancy in three conifers. Tree Physiol. 5:291-306.

Camm, E.L., and Harper, G.J. 1991. Temporal variations in cold sensitivity of root growth in cold-stored white spruce seedlings. Tree Physiol. 2:425-431.

Camm, E.L., Harper, G.J., Rosenthal, S.I., and Camm, D.M. 1993. Effect of photon flux density on carbon assimilation and chlorophyll a fluorencence of cold-stored white spruce and lodgepole pine seedlings. Tree Physiol. 12:185-194.

Camm, E.L., Goetze, D.C., Silim, S.N., and Lavender, D.P. 1994. Cold storage of conifer seedlings: An update from the British Columbia perspective. For. Chron. 70:311-316.

Camm, E.L., Guy, R.D., Kubien, D.S., Goetze, D.C., Silim, S.N., and Burton, P.J. 1995. Physiological recovery of freezer-stored white and Engelmann spruce seedlings planted following different thawing regimes. New For. 10:55-77.

Cannell, M.G.R., Tabbush, P.M., Deans, J.D., Hollingsworth, M.K., Sheppard, L.J., Philipson, J.J., and Murray, M.B. 1990. Sitka spruce and Douglas-fir seedlings in the nursery and in cold storage: root growth potential, carbohydrate content, dormancy, frost hardiness and mitotic index. Forestry, 63:9-27.

Carlson, W.C. 1985. Effect of natural chilling and cold storage on budbreak and root growth potential of loblolly pine (*Pinus taeda* L.). Can. J. For. Res. 15:651-656.

Forry, K.R., and Zaerr, J.B. 1988. The starch content of roots as an estimator of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedling quality. Northwest. Sci. 62:70 (Abstr.).

Jiang, Y., Zwaizek, J.J., and Macdonald, S.E. 1994. Effects of prolonged cold storage on carbohydrate and protein content and field performance of white spruce bareroot seedlings. Can. J. For. Res. 24:1369-1375.

Jiang, Y., Macdonald, S.E., and Zwiazek, J.J. 1995. Effects of cold storage and water stress on water relations and gas exchange of white spruce (*Picea glauca*) seedlings. Tree Physiol. 15:267-273.

Levesque, F. 1996. Carbohydrates in white spruce and lodgepole pine seedlings during winter, outdoors, in freezer storage and thawing. M.Sc.Thesis, University of British Columbia. Vancouver, 131 pp.

Marshall, J.D, and Waring, R.H. 1985. Predicting fine roots production and turnover by monitoring root starch and soil temperature. Can. J. For. Res. 15:791-800.

Mattsson, A., and Troeng, E. 1986. Effects of different overwinter storage regimes on shoot g rowth and net photosynthetic capacity in *Pinus sylvestris* seedlings. Scand. J. For. Res. 22:75-84.

McCraken, I.J. 1979. Changes in the carbohydrate concentration of pine seedlings after cool storage. N.Z.J. For. Sci. 9:34-43.

Omi, S.K., Rose, R., and Sabin, T.E. 1994. Fall lifting and long-term freezer storage of ponderosa pine seedlings: effects on starch, root growth, and field performance. Can. J. For. Res. 24:624-637.

Puttonen, P. 1986. Carboyhdrate reserves in *Pinus sylvestris* seedling needles as an attribute of seedling vigor. Scand. J. For. Res. 1:181-193.

Ritchie, G.A. 1982. Carbohydrate concentration reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Can. J. For. Res. 12:905-912.

Ritchie, G.A. 1987. Some effects of cold storage on seedling physiology. Tree Plant. Notes, 38:11-15.

Ronco, F. 1973. Food reserves of Engelmann spruce planting stock. For. Sci. 19:213-219.

Stone, E.C.,and Jenkenson, J.L. 1970. Influence of soli water on root growth capacity of Ponderosa pine transplants. For. Sci. 16: 230-239.

Tabbush, P.M. 1988. Silvicultural practices for plant restocking. Fom. Comm. Bull. (U.K.) 76.

Troeg, E. 1991. Photosynthesis of root respiration of *Pinus sylvestris* and *Picea abies* seedlings after different root freezing and storage temperatures. Scand. J. For. Res. 6:53-58.

Venn, K. 1980. Winter vigour in *Pices abies* (L.) Karst. VII. Development of injury to seedlings during over winter cold storage. A literature review. Rep. Norw. For. Res. Inst. 35.9.

Vernon, 1960. Spectrophotometric determination of chlorophyll and pheophytins in plant extracts. Anal. Chem. 32:1144-1150.

CHAPTER V

5. *In vivo* STUDY OF LODGEPOLE PINE SEEDLING DROUGHT TOLERANCE

5.1 Introduction

Drought tolerance at the time of planting is an important factor influencing seedling survival and growth (Blake and Sutton, 1987; Kozlowski et al., 1991). Newly planted seedlings, regardless of how carefully handled, will almost always be subjected to some degree of water deficit. This is particularly true since the seedlings often have a very small root system at planting (Sands, 1984). Therefore, any period of drought can quickly result in severe water stress and high mortality as a result of poor water uptake by the roots.

Loss of cell membrane integrity is associated with drought stress and can be measured as electrolyte leakage (Levitt, 1980). Electrolyte leakage has been shown to be proportional to the level of drought injury in winter rape (*Brassica napus*) (Dlugokecka and Kacperska-Palacz, 1978). Both Zwaizek and Blake (1990) and Martin et al. (1987) used electrolyte leakage to measure drought stress in conifers. Zwaizek and Blake (1990) noted that electrolyte leakage was superior to electrical impedance and xylem sap osmolarity methods in detecting drought stress-induced membrane damage. Electrolyte leakage is measured as a percent of the total electrolytes and is then referred to simply as injury (Blum and Ebercon, 1981). It has also been suggested as a possible indicator of seedling performance in the absence of drought (McKay and Mason, 1991).

Plants can be preconditioned to tolerate drought stress by subjecting them to moderate levels of drought (Levitt, 1980). This induces a number of changes, including increased stomatal resistance and the accumulation of osmotically active solutes (Ackerson and Herbert, 1981), including monosaccharides and disaccharides (Martin et al., 1987). van den Driessche (1991) demonstrated that lodgepole pine seedlings that had been exposed to drought cycles in the nursery retained tolerance even after outplanting. This, however, depended on the storage conditions. Jiang et al. (1994; 1995) noted that storage conditions resulting in

depletion of storage reserves (starch and soluble carbohydrates) resulted in reduced drought tolerance.

Despite the fact that lodgepole pine is frequently planted on dry sites and, therefore, drought tolerance would be advantageous, there has been little research on *in vivo* assessment of drought tolerance in this species. The objective of the present study was to develop the methodology for *in vivo* assessment in lodgepole pine. Once this was accomplished, the relationships between seedling carbohydrate status and stress tolerance, and stress tolerance and outplanting performance were assessed. Finally, the potential to use MC (moisture content) or RGC after stress or carbohydrate analysis prior to stress to predict drought stress tolerance was addressed, using electrolyte leakage as the indicator of drought stress injury.

5.2. Material and Methods

5.2.1 Plant Material

Lodgepole pine (*Pinus contorta* Dougl. spp. *latifolia*) seedlings (seedlot #30862: 1500 m elevation; 50°54'N; 121°08'W) were lifted from the greenhouse (Pitt Meadows, British Columbia) on September 19, October 16, and 31, November 14, December 01, and 12, 1995 (Chapter III, Table 3.1) and September 23, November 14, and December 06, 1996 (Table 5.1). Assessments were made prior to and after cold storage.

Table 5.1: Lifting dates.

Lifting Dates	Number of days in cold storage	Assessment Dates
September 23, 1996	158	February 28, 1997
November 14, 1996	121	March 15,1997
December 06, 1996	131	April 16, 1997

5.2.2 Development of Drought Stress Experimental Design

A sample of 120 seedlings was selected from a pool of seedlings from all six lifting dates prior to cold storage in 1995. Seedlings were planted individually in 95 mm by 230 mm pots containing sand-peat-vermiculite (2:1:1, v/v/v), and grown under controlled conditions of 22°C with a 16 h photoperiod of 400 µmol $m^{-2}s^{-1}$ photon flux density and 75% relative humidity. Control seedlings (36) were watered to the point of runoff on days 0, 1, 4 and 8. These were considered to be optimum conditions. Drought stressed seedlings (36) were planted into dry substrate and were not watered during the 10 day drought stress treatment. Soil moisture content (MC) and needle moisture content from the control group were determined on 6 separate samples on each of day 0, 2, 4, 6, 8, 10. The seedlings from the stressed group (n=6) were used for determination of needle MC, needle injury and root injury, after 0, 2, 4, 6, 8, 10 days of drought stress. The remaining 48 seedlings were used to determine RGC after 0, 2, 4, 6, 8, 10 days of drought (n=8).

5.2.3 Pre-storage Experiment

Seedlings (20) were lifted on 3 lifting dates in 1996, and subjected to a 10 day drought stress as described above. Needle MC, needle injury and root injury, RGC and survival rate were determined on day 10.

5.2.4 Post-storage Experiment

Seedlings (20) were lifted on each lifting date, cold stored at -2°C and transplanted as described in Chapter III; Chapter IV (Materials and Methods, 3.2.3; 4.2.3). After storage, the seedlings were thawed at 4°C in a cold room for 7 days. The seedlings from 6 lifting dates in 1995 and 3 lifting dates in 1996 were submitted to a 10 day drought stress. The same parameters were monitored as above.

5.2.5 Moisture Content of Soil (MC)

On day 0, and every second day thereafter, 1 g soil samples (1 from each pot) were taken approximately 5 cm from the soil surface, weighed, and dried for 2 days at 60°C (until constant weight) and reweighed. The moisture content was expressed as:

$$MC\ (\%) = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100 \qquad (Equation\ 5.1)$$

5.2.6 Content of Needles (MC)

Approximately 0.5 g of needles from each of 6 seedlings were taken from 20 mm below the apical bud. Samples were weighed, dried, reweighed and the MC calculated as above (Tabbush, 1987).

5.2.7 Electrolyte Leakage of Needles

Electrolyte leakage was measured in needle samples taken 30 mm below the apical bud as follows. Approximately 0.3 g of needles cut in 1 cm long pieces from 6 control and 6 stressed seedlings were placed individually into 28 mL glass bottles containing 16 mL distilled water of a known conductivity. The bottles were capped and left at room temperature for 24 h and the conductivity was measured on CDM 83 conductivity meter (Radiometer, Copenhagen, Denmark). Following autoclaving for 10 min at 120°C, cooling to room temperature and adjusting the volume, the total conductivity of each sample was measured (McKay, 1992). Stress was expressed as percent injury, calculated (Blum and Ebercon, 1981) as:

$$\text{percent injury} = 1-[1-(T_1/T_2)/1-(C_1/C_2)] \times 100 \qquad (Equation\ 5.2)$$

where:

T and C - refer to the means of treatment and control, respectively, and subscripts $_1$ and $_2$ - refer to the initial and final conductivities, respectively.

5.2.8 Electrolyte Leakage of Roots

The roots of control (6) and stressed (6) seedlings were washed in cold tap water to remove soil and rinsed in deionized water to remove surface ions. The central root mass of each seedling was sampled by removing a 20 mm wide band of roots at a distance of approximately 80-100 mm from the root collar. Roots less than 2 mm in diameter were randomly collected from the band. The sample size was approximately 0.3 g. Electrolyte leakage was measured and percent injury determined as above.

5.2.9 Root Growth Capacity (RGC)

Seedlings (8) were subjected to a 10 day drought stress. Starting on day 11, the seedlings were watered to the point of runoff every second day and were grown under optimum conditions (Chapter III, Materials and Methods, 3.2.5) for 7 days to estimate RGC (Johnson-Flanagan and Owens, 1985). Control seedlings (8) were also subjected to the RGC test.

5.2.10 Survival Rate

Seedling survival was determined on the basis how many seedlings were able to grow new roots in 7 days under RGC conditions after they were exposed to a 10 day drought stress. Eight seedlings were sampled for each lifting date.

5.2.11 Statistical analysis

Simple linear correlations were computed to examine the relationship between both needle MC and needle injury and root injury and RGC over a 10 day stress period. The relationships between needle MC and needle injury, and RGC and root injury, in addition to their relationship with field performance were examined using a simple linear correlation. Simple linear correlations were applied to examine relationships between both RGC and root injury and root reducing, total soluble, and total non-structural carbohydrates, and starch.

Differences in needle MC and needle injury, RGC and root injury after cold storage, among 6 lifting dates in 1995 and prior to and after storage among 3 lifting dates in 1996 were determined with sources of variation of LD and experimental error using one-way ANOVA. Specific differences among LD means were determined with the least significant difference (LSD) test. All data parameters in 1996 listed above were analyzed by two-way ANOVA (lifting dates versus treatments prior to and after cold storage). A significance level of $p<0.05$ was used. Compilation was done using the General Linear Model of SAS 6.10 (SAS Institute Inc., Carry, NC 27513, USA).

5.3 Results

5.3.1 Preliminary Study

The results of substrate MC presented in Table 5.2 show that the well watered (control) substrate contained 28-31% moisture. The average moisture content used for drought stress was approximately 10% on day zero, dropping by day 10 to 1.0%. The results indicate a water deficit in the substrate. The method was the same as that used by Marsden et al. (1996) to simulate drought conditions. The seedlings from the control group had an average needle MC of 65% over 10 days (Table 5.2). Needle MC decreased linearly to 53% by day 10 in drought stressed seedlings. This indicates that the seedlings were experiencing drought conditions.

Electrolyte leakage of needles is expressed as percent injury caused by drought stress. There was a linear increase in needle injury from 2.6% on day 2 to 18% on day 10 (Table 5.2). Needle injury and MC were related in an inverse linear manner (r^2=-0.97; $p<0.001$). A more dramatic increase in injury during 10 days of drought stress was observed in roots (Table 5.2). The injury increased linearly from 4.7% on day 2 to 42% on day 10.

The total number of white roots produced in the RGC test decreased linearly from 115 to 20 during 10 days of stress (Table 5.2). Root injury and RGC showed a highly significant inverse linear correlation (r^2=-0.96; $p<0.001$).

Despite a large increase in drought stress injury (measured as needle and root electrolyte leakage) over the 10 day period, all seedlings survived the stress treatment measured after 7 day regrowth. The results from this preliminary drought stress experiment indicated that a 10 day drought stress treatment was suitable to use for further experimentation.

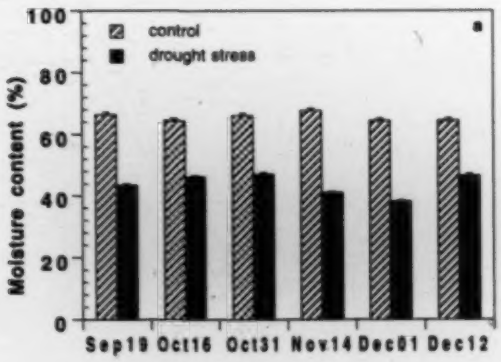
5.3.2 1995 Study

Seedlings were exposed to the 10 day drought stress treatment following cold storage and thawing. The control seedlings showed an average needle MC of 65% (Fig. 5.1a); the same as for seedlings that had not been in cold storage (Table 5.2).

Table 5.2: Parameters tested in order to determine the drought stress regime.

Stress parameter	Day of stress 0	2	4	6	8	10	Linear regression equations for drought conditions
MC of substrate (%)	10.3 ± 0.3*	5.9 ± 0.3	4 ± 0.1	3.1 ± 0.07	1.6 ± 0.2	1.1 ± 0.07	y=10.35-1.72x r^2= 0.94
Control	29.7 ± 0.4	28.6 ± 0.5	29.5 ± 0.4	28.3 ± 0.2	30.2 ± 0.3	31.2 ± 0.3	
MC of needle (%)	67 ± 1*	63 ± 1.2	60 ± 1.8	57 ± 1.6	55 ± 1.8	53 ± 2.9	y=68.56-2.67x r^2= 0.99
Control	65 ± 1	66 ± 0.8	63 ± 0.7	62 ± 0.7	65 ± 1	66 ± 1.3	
% of needle injury	0*	2.6 ± 0.4	7 ± 1.2	10 ± 1	13 ± 2.9	18 ± 1.6	y=-12.4+9.09x r^2= 0.98
% of root injury	0*	5 ± 1	11 ± 1	21 ± 3	36 ± 3	43 ± 4	y=-3.99+3.55x r^2= 0.99
RGC	115 ± 6**	112 ± 10	95 ± 9	54 ± 5	41 ± 9	20 ± 5	y=146.1-20.7x r^2= 0.97

mean± SE ;* n=6 seedlings ; ** n=8 seedlings; MC, moisture content



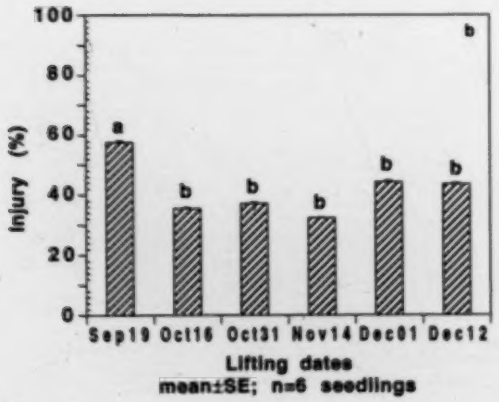


Figure 5.1: Response of lodgepole pine seedling needles to drought stress following cold storage in 1995

a) Moisture content in needles from control and drought stressed seedlings. No significant effects detected by a one-way ANOVA.
b) Percent needle injury in response to drought stress. Means having common letter(s) are not significantly different ($p>0.05$) according to a one-way ANOVA and LSD test.

Needle MC of seedlings that had been drought stressed for 10 days varied from 38% to 47%, depending on the lifting date. The highest needle MC was in seedlings lifted on Oct. 31, 1995; the lowest value was in seedlings lifted on Dec. 01, 1995, but the differences were not significant (LSD, $p<0.05$).

The response of needles to drought stress following cold storage was assessed by electrolyte leakage measurements (Fig. 5.1b). The values, expressed as percent injury, varied from 32% (Nov. 14, 1995) to 58% (Sep. 19, 1995). Seedlings lifted on Sep. 19, 1995 had the highest percent injury (LSD, $p<0.05$). No other significant differences were noted. Percent injury was not significantly correlated with needle MC (r^2=-0.01, $p<0.8$).

Root injury changed significantly (LSD, $p<0.05$) over the study period. Injury decreased from Sep. 19 to Oct. 31, remained low on Nov. 14, [both Oct. 31 and Nov. 14 had significantly lower (LSD, $p<0.05$) root injury in comparison to other dates] and increased thereafter (Fig. 5.2a). Root injury ranged from a low of 31% (Nov. 14, 1995) to a high of 66% (Dec. 12, 1995).

Control seedlings lifted on Nov. 14, 1995 had the highest number of white roots following RGC (Fig. 5.2b) while seedlings lifted on Sep. 19, 1995, had the lowest number. Drought conditions significantly decreased (LSD, $p<0.05$) the RGC of cold-stored seedlings. Following stress, the highest the number of white roots produced in the RGC test was in seedlings lifted on Nov. 14, 1995; the lowest number was in seedlings lifted on Sep. 19, 1995, with the average value being 25 white roots. RGC and root injury showed a significant inverse correlation (r^2=-0.68; $p<0.05$).

Root architecture as well as root growth is important for survival. Following drought stress, root growth following RGC was confined to the bottom and middle region of the root system (Fig. 5.2c). In comparison, cold-stored seedlings that were not exposed to drought stress (control seedlings) had white roots on the top region of the root system and proportionately more white roots in the middle region (Fig. 3.2b).

Survival results (Table 5.3) indicate that seedlings lifted on Sep. 19, 1995, were the least able to survive the stress. Seedlings collected on Oct. 31, and Nov. 14, 1995 had 100% survival.

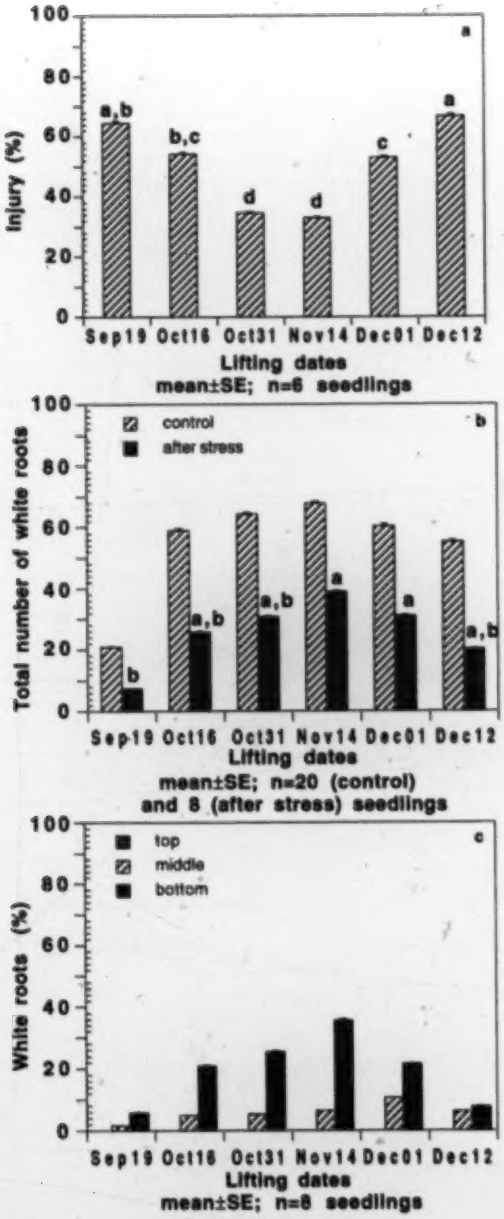


Figure 5.2: Response of lodgepole pine seedling roots to drought stress following cold storage in 1995

a) Percent root injury in response to drought stress. Means having common letter(s) are not significantly different ($p>0.05$) according to a one-way ANOVA and LSD test.
b) RGC of control and drought stressed seedlings. Statistics are as described for Fig. 5.2a.
c) Percent of white roots in different regions of the root system under RGC conditions following drought stress. Data were not analyzed by ANOVA.

Drought tolerance is expected to be controlled, in part, by carbohydrate content (Thomas 1990; Zwiazek and Blake, 1990). RGC following drought stress showed a significant correlation with reducing ($r=0.87$; $p<0.05$), total soluble ($r=0.86$; $p<0.05$) and total non-structural carbohydrate ($r=0.86$; $p<0.05$) following storage (Table 5.4). At the same time, root injury was significantly correlated in an inverse manner with total soluble carbohydrate ($r=-0.84$; $p<0.05$) and total non-structural carbohydrate ($r=-0.81$; $p<0.05$) following storage.

Table 5.3: Survival rate of seedlings (following storage) after a 10 day drought stress and regrowth for 7 days under RGC conditions.

Lifting Dates	Survival rate (%)
September 19, 1995	50
October 16, 1995	62.5
October 31, 1995	100
November 14, 1995	100
December 01, 1995	62.5
December 12, 1995	62.5

n=8 seedlings

Table. 5.4: Correlation coefficients (r) for RGC and root injury with root reducing carbohydrate, total soluble carbohydrate, starch and total non-structural carbohydrate following cold storage and drought stress in 1995.

Stress parameter	Reducing carbohydrate	Total soluble carbohydrate	Starch	Total non-structural carbohydrate
RGC	0.87 (0.02)	0.86 (0.03)	0.73 (0.1)	0.86 (0.03)
Root injury	- 0.62 (0.2)	- 0.84 (0.03)	- 0.52 (0.3)	- 0.81 (0.03)

p values are indicated in brackets, n=8

In order to determine whether drought tolerance had an impact on seedling growth following outplanting, correlations were carried out between the indices of stress and field performance (Table 5.5). Neither MC of stressed needles nor root injury correlated significantly with any measured field parameter. RGC following drought stress was correlated with field survival ($r=0.86$; $p<0.03$) and collar diameter ($r=0.86$; $p<0.03$). Needle injury showed the highest inverse correlation with field survival ($r=-0.89$; $p<0.01$), and with height increment in 1996 ($r=-0.94$; $p<0.01$). Needle injury was also inversely correlated with collar diameter ($r=-0.81$; $p<0.05$).

5.3.3 1996 Study

Three lifting dates were studied in 1996. The results for 1996 were similar to those for 1995. In both years, seedlings that had not collected chilling hours (Sep. samples) performed poorly and those lifted soon after the chilling requirement had been met (mid-Nov.) performed well.

In 1996, the control seedlings had an average of 59% and 63% needle MC, prior to and after storage, respectively (Fig. 5.3a). Needle MC from drought stressed seedlings was an average of 45% and 52%, prior to and after storage, respectively. Needle MC of seedlings lifted on Sep. 23, 1996, prior to storage, was significantly lower (LSD, $p<0.05$) than that following storage. There was no significant effect of storage on the other dates. There was a significant interaction between needle MC prior to and after cold storage and lifting date ($p<0.001$).

There was a good inverse correlation between needle MC and injury prior to ($r^2=-0.97$; $p>0.1$) and after storage ($r^2=-0.87$; $p>0.2$). Seedlings lifted on Sep. 23, 1996 had significantly higher (LSD, $p<0.05$) needle injury (45% and 36%), prior to and after storage in comparison with the other dates (Fig. 5.3b). The lowest needle injury was in seedlings lifted on Nov. 14, 1996 (12% and 7%, prior to and after storage, respectively). No significant interactions occurred between needle injury prior to and after cold storage and lifting date ($p>0.2$).

Root injury followed the same trend as needle injury (Fig. 5.4a). Root injury for

Table 5.5: Correlation coefficients (r) between drought stress parameters measured after cold storage and field performance for lodgepole pine seedlings lifted in 1995.

Stress parameter	Survival	Collar diameter	Most distal needle length	Height at planting	Increment in 1996	Total height
Needle moisture content	0.02 (0.9)	- 0.18 (0.7)	- 0.25 (0.6)	- 0.39 (0.4)	0.22 (0.7)	- 0.21 (0.7)
Needle injury	- 0.89 (0.02)	- 0.81 (0.05)	- 0.62 (0.2)	-0.26 (0.6)	- 0. 94 (0.001)	- 0.66 (0.1)
RGC after drought stress	0.86 (0.03)	0.86 (0.03)	0.74 (0.1)	0.45 (0.4)	0.73 (0.1)	0.75 (0.1)
Root injury	- 0.53 (0.3)	- 0.53 (0.3)	- 0.28 (0.6)	- 0.07 (0.9)	- 0.51 (0.3)	- 0.30 (0.6)

p values are indicated in brackets, n=6

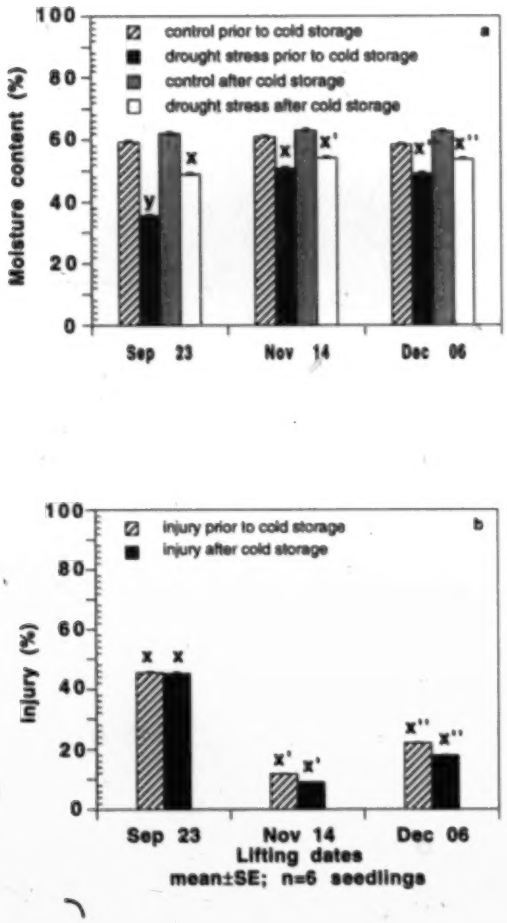


Figure 5.3: Response of lodgepole pine seedling needles to drought stress prior to and after cold storage in 1996

a) Moisture content of stressed needles prior to and after cold storage in 1996. Means having common letter(s) are not significantly different ($p>0.05$) at a lifting date according to a one-way ANOVA and LSD test.
b) Percent needle injury in response to drought stress prior to and after storage in 1996. Statistics are as described for Fig. 5.3a.

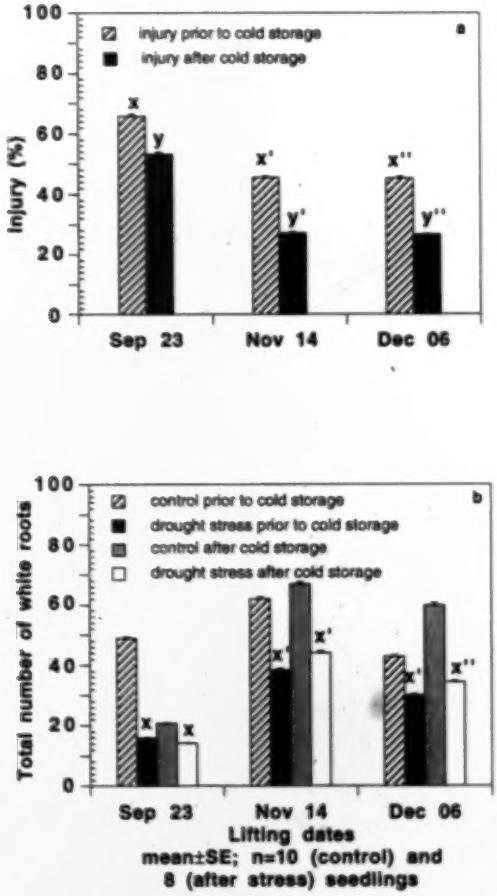


Figure 5.4: Response of lodgepole pine seedling roots to drought stress prior to and after cold storage in 1996

a) Percent root injury in response to drought stress prior to and after cold storage. Means having common letter(s) are not significantly different ($p>0.05$) at alifting date according to a one-way ANOVA and LSD test.

b) RGC of control and drought stressed seedlings prior to and after cold storage in 1996. Statistics are as described for Fig. 5.4a.

seedlings lifted in September was significantly higher (LSD, $p<0.05$) than in seedlings lifted in November or December, prior to and after storage. Seedlings lifted on all 3 lifting dates had significantly higher LSD, $p<0.05$) root injury prior to storage than after cold storage. No significant interactions occurred between root injury prior to and after cold storage and lifting date ($p>0.3$).

In 1996, RGC was correlated with root injury after storage (r^2=-0.81; $p>0.3$), but the correlation was poor prior to storage (r^2=-0.06; $p>0.8$). The highest number of white roots was observed in seedlings lifted on Nov. 14, 1996, prior to and after storage, respectively (Fig. 5.4b). The lowest number of white roots was in seedlings lifted on Sep. 23, 1996, prior to and after storage, respectively. Cold storage did not alter the effect of drought stress on RGC. No significant interactions occurred between RGC prior to and after cold storage and lifting date ($p>0.7$). In general, root growth was confined to the bottom and middle region of the root system (Fig. 5.5a,b). Only seedlings lifted on Nov. 14, 1996 had white roots in the top of the root system.

Seedlings lifted on Sep. 23, 1996 were least able to survive drought (Table 5.6). Again, seedlings lifted on Nov. 14, 1996, had the highest survival prior to and after storage. In general, storage improved seedling survival of drought stress.

There was a significant inverse correlation between root injury and both total soluble carbohydrate (r=-0.84; $p<0.05$) and total non-structural carbohydrates (r=-0.81; $p<0.05$) in the 1996 post-storage seedlings (Table 5.7).

5.3.4 Statistical Analysis of 1995 and 1996 Results

Results from the preliminary study suggested that correlations existed between needle MC and needle injury, and RGC and root injury. Needle MC and needle injury were not significantly correlated in the 1995 data set (r^2=-0.01; $p>0.8$); increasing the sample size to n=9 by combining the post-storage data from 1995 and 1996 resulted in a significant correlation (r^2=-0.51; $p<0.05$).

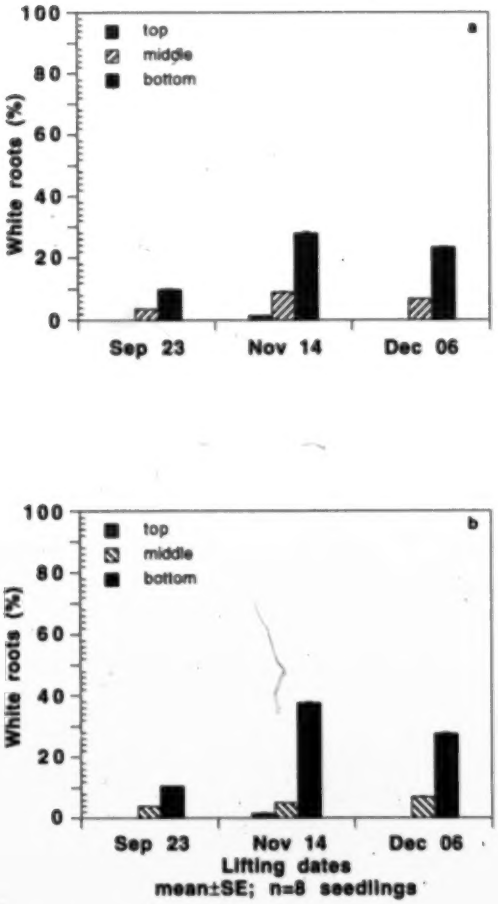


Figure 5.5: Response of lodgepole pine seedling roots to drought stress

a) Percent of white roots in different regions of the root system under RGC conditions following drought stress prior to cold storage in 1996. Data were not analyzed by ANOVA.
b) Percent of white roots in different regions of the root system under RGC conditions following drought stress after cold storage in 1996. Data were not analyzed by ANOVA.

Table 5.6: Survival rate of seedlings (prior to and after storage) after a 10 day drought stress and regrowth for seven days under optimal conditions.

Lifting Dates	Survival rate (%)
September 23, 1996	
prior to storage	50
after storage	62.5
November 14, 1996	
prior to storage	100
after storage	100
December 06, 1996	
prior to storage	87
after storage	100

n=8 seedlings

Table. 5.7: Correlation coefficients for RGC and root injury with root reducing carbohydrate, total soluble carbohydrate, starch and total non-structural carbohydrate following cold storage and drought stress in 1996.

Stress parameter	Reducing carbohydrate	Total soluble carbohydrate	Starch	Total non-structural carbohydrate
RGC	0.33 (0.2)	0.47 (0.3)	0.16 (0.7)	0.48 (0.3)
Root injury	- 0.14 (0.8)	- 0.84 (0.03)	- 0.52 (0.3)	- 0.81 (0.03)

p values are indicated in brackets

Similarly, the preliminary study showed that RGC and root injury after drought stress were significantly correlated. Following cold storage, RGC and root injury after drought stress were significantly correlated in 1995 (r^2=-0.68; $p<0.05$). This correlation was strengthened by combining two years post-storage data (r^2=-0.68; $p<0.01$). In order for a parameter to

have general applicability for screening purposes, the regression equations for each year must be similar (McKay, 1992). As the slopes of the regression lines were similar (Table 5.8), this indicates that RGC following drought stress may be useful as a general measure of drought stress tolerance following cold storage.

RGC and root injury were significantly inversly correlated with total soluble and total non-structural carbohydrates in 1995 ($r=0.86$, $p<0.03$; $r=-0.86$, $p<0.03$). In 1996, there was a significant inverse correlation between root injury and total soluble and total non-structural carbohydrates ($r=-0.84$, $p<0.05$; $r=-0.81$, $p<0.05$). RGC was not significantly correlated with either total soluble or total non-structural carbohydrates in 1996.

Table 5.8: Linear regression equations of RGC and root injury after drought stress in 1995 and 1996 following drought stress after cold storage.

Year	Linear regression equation
1995	RGC = 78.8 - 1.08 injury, ($r^2 = 0.67$)
1996	RGC = 64.4 - 0.96 injury, ($r^2 = 0.81$)

The linear regression equations of root injury with total soluble and total non-structural carbohydrates in 1995 and 1996 after storage are presented in Table 5.9. Comparison between the regression equations failed to show any similarity between data for 1995 and 1996, indicating, therefore, that total soluble and total non-structural carbohydrates can only be used as relative predictors of root injury.

5.4 Discussion

The results from the present study indicate that cold storage induces drought stress tolerance in lodgepole pine seedlings. All parameters measured, including needle injury, root injury, RGC and seedling survival improved on most lifting dates following storage. These results agree with those of Blake (1983) who attributed increased avoidance to the effect to

reduced stomatal opening. The findings of the present study support this, as needle MC was higher following cold storage, both before and after drought stress.

Table 5.9: Linear regression equations of root injury with total soluble and total non-structural carbohydrates in 1995 and 1996 following drought stress after cold storage.

Parameter	Year	Linear regression equation	
		Total soluble carbohydrate	Total non structural-carbohydrate
Root injury	1995	Injury = 83.7 - 3.39 TSCH (r^2 = 0.71)	Injury = 81.2 - 2.29 TNSCH (r^2 = 0.62)
Root injury	1996	Injury = 1.65 - 4.3 TSCH (r^2 = 0.65)	Injury = 1.11 - 4.41 TNSCH (r^2 = 0.65)

* TSCH - total soluble carbohydrate; ** TNSCH - total non structural carbohydrate

Reduced water usage does not entirely explain the increase in drought tolerance. In the present study, the correlation between needle MC and needle injury was inconsistent. Jiang et al. (1995) noted that increased stress tolerance after cold storage was not a result of reduced stomatal conductance. These results indicate that factors other than water usage contribute to cold storage-induced increases in drought tolerance. Perhaps solute accumulation or allocation is involved.

While Smit-Spinks et al. (1985) found that aerial tissue was capable of hardening to a far greater degree than were roots, in the present study, measurements of electrolyte leakage indicated that the cold storage increased drought tolerance to a greater extent in roots than in needles. This difference aside, the roots are certainly more sensitive to a given level of drought stress, both before and after cold storage. These results indicate that root injury due to drought stress is still be a major concern in the production of seedlings for reforestation.

The relationship between root carbohydrates and drought stress in conifers has received considerable attention. Jiang et al. (1994) reported that high drought tolerance (measured as

water potential) was associated with high starch content. They found that starch content yielded more soluble sugars during drought and this altered the osmotic potential in favour of reduced plasmolysis under stress conditions (Jiang et al., 1995). On the other hand, Zwaizek and Blake (1990) showed that turgor maintenance was largely explained by the accumulation of soluble sugars. Munns and Wier (1981) and Thomas (1990) also suggested that soluble sugars contributed to enhanced drought tolerance, measured as root growth after drought stress. The results from the present study indicate that total soluble carbohydrate and total non-structural carbohydrate are inversely correlated to root injury, and starch content is not correlated in any way. On the basis of these findings, there appears to be a strong inverse relationship between both total soluble carbohydrate content and total non-structural carbohydrate content and root drought tolerance.

One objective of this study was to determine if drought stress tolerance indices could be developed on the basis of easily measured parameters, such as MC and RGC after drought stress. In order to be useful, the correlations between these and drought stress tolerance, measured as needle or root injury, must be consistent from year to year. Although MC was significantly correlated with needle injury in the preliminary study, where drought stress increased over time, the results over the two years were not consistent. In addition to indicating that MC is not a good measure of seedling drought tolerance, this demonstrates that care must be taken when extrapolating results from time course experiments.

Results for post-storage RGC after stress as an indicator of drought stress are better. The correlation between RGC and root injury after drought stress was highly significant in the preliminary study, significant in 1995, good in 1996, with a high r^2 value (0.81) and highly significant for the combined 1995 and 1996 data (r^2=0.68, $p<0.01$). For a parameter to be a universal measure rather than a relative measure, the regression equations must be similar from year to year (McKay, 1992). As the equations were similar, RGC of post-storage seedlings after drought stress may be useful as a general measure of drought stress tolerance in any given year. It should be noted, however, that injury assessments using electrolyte leakage are faster and require less equipment and space.

An alternative approach is to predict drought tolerance. The present study examined root carbohydrate content in this regard. In both years, drought stress tolerance as indicated by electrolyte leakage after cold storage was significantly correlated with total soluble carbohydrate and total non-structural carbohydrate. On this basis, it would be appear that total soluble carbohydrate or total non-structural carbohydrate content are good predictors of drought stress tolerance. However, they would only be useful as relative measures (see Table 5.9).

The ultimate objective of any study of drought stress tolerance in conifer seedlings is to relate the findings to field survival and seedling growth after outplanting. Jiang et al. (1994) found that low drought tolerance, measured as low water potential, resulted in reduced root growth initially, but no decrease in survival or growth over the season. Their work was on white spruce, and the outplanting sites were in northern Alberta, whereas our study involved lodgepole pine outplanted into the dry environment of the Okanagan Vally in British Columbia. Therefore, drought stress tolerance may not have been a factor in determining survival following outplanting. The results from the present study indicate that drought stress tolerance does lead to better survival and growth after outplanting. The present study also shows that needle injury and RGC after drought stress in post-storage seedlings are the best indicators of subsequent survival and growth. Of these, needle injury was the better measure, having a highly significant correlation with height increment and significant correlations with both survival and collar diameter. The general applicability of these measurements as predictors of outplanting success and growth will be determined in the coming year.

In summary, cold storage increased drought stress tolerance in lodgepole pine seedlings. The increase in tolerance in the roots was associated with increases in root total soluble carbohydrate and total non-structural carbohydrate. In fact, the results strongly suggest that total soluble carbohydrate and total non-structural carbohydrate following cold storage are excellent predictors of drought tolerance. In terms of outplanting success and subsequent growth, needle injury and RGC after stress were suitable indicators, with needle injury being superior. As these assessments were completed at least one month prior to outplanting, there is plenty of time to make the necessary decisions regarding outplanting.

5.5 Literature Cited

Ackerson, R.C., and Herbert, R.R. 1981. Osmoregulation in cotton in response to water stress. I. Alterations in photosynthesis, leaf conductance, translocation, and ultrastructure. Plant Physiol. 67:484-488.

Blake, R.J. 1983. Transplanting stress in white spruce: Effect of cold storage and root pruning on water relations and stomata conditioning. Physiol. Plant. 57:210-216.

Blake, T.J., and R.F. Sutton.1987. Variation in water relations of black spruce stock types planted in Ontario. Tree Physiol. 3:331-344.

Blum, A., and Ebercon, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21:43-47.

Dlugokecka, E., and Kacperska-Palacz, A. 1978. Re-examination of electrical conductivity method for estimation of drought injury. Biol. Plant. 20:262-267.

Jiang, Y., Zwaizek, J.J., and Macdonald, S.E. 1994. Effects of prolonged cold storage on carbohydrate and protein content and field performance of white spruce bareroot seedlings. Can. J. For. Res. 24:1369-1375.

Jiang, Y., Macdonald, S.E., and Zwiazek, J.J. 1995. Effects of cold storage and water stress on water relations and gas exchange of white spruce (*Picea glauca*) seedlings. Tree Physiol. 15:267-273.

Johnson-Flanagan, A.M., and Owens, J. N. 1985. Root growth and root growth capacity of white spruce (*Picea glauca* [Moench] Voss) seedlings. Can. J. For. Res. 15:625-630.

Kozlowski, T.T., Kramer, P.J., and Pallardy, S.G. 1991. The physiological ecology of woody plants. Academic Press, Toronto, 657p.

Levitt, J. 1980. Responses of plants to environmental stresses. Academic Press, New York, pp. 497.

Marsden, B.J., Lieffers, V.J., and Zwiazek, J.J. 1996. The effect of humidity on photosynthesis and water relations of white spruce seedlings during the early establishment phase. Can. J. For. Res. 26:1015-1021.

Martin, U., Pallardy, S.G., and Bahari, Z.A. 1987. Dehydratation tolerance of leaf tissues of six woody angiosperm species. Physiol. Plant. 69:182-186.

McKay, H. M. 1992. Electrolyte leakage from fine roots of conifer seedlings: a rapid index of plant vitality following cold storage. Can. J. For. Res. 22:1371-1377.

McKay, H.M., and Mason, W.L. 1991. Physiological indicators of tolerance to cold storage in Sitka spuce and Douglas-fir seedlings. Can. J. For. Res. 21:890-901.

Munns, R., and Weir, R. 1981. Contribution of sugars to osmotic adjustment in elongated and expanded zones of wheat leavs during moderate water deficits at two light leves. Aus. J. Plant. Physiol. 8:93-105.

Sands, R. 1984. Transplanting stress in radiata pine. Aust. For. Res. 14:67-72.

Smit-Spinks, B., Swanson, B.T., and Muckhart, A.H. 1985. The effect of photoperiod and thermoperiod on cold accumulation and growth on *Pinus sylvestris*. Can. J. For. Res. 15:453-460.

Tabbush, P. M. 1987. Effect of desiccation on water status and forest performance on bare-root Sitka spruce and Douglas fir transplants. Forestry, 60:31-43.

Thomas, H. 1990. Osmotic adjustment in *Lolium perene*; it heritability and the nature of solute accumulation. Ann. Bot. 66:521-530.

van den Driessche, R. 1991. Influence of container nursery regimes on drought resistance of seedlings following planting. I. Survival and growth. Can. J. For. Res. 21:555-565.

Zwiazek, J.J. and T.J. Blake. 1990. Effects of preconditioning on carbohydrate and amino acid composition of osmotically stressed black spruce (*Picea mariana*) cuttings. Can. J. For. Res. 20:108-112.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Over the years, seedling nurseries and forest companies have become increasingly aware that the industry needs to focus on seedling growth and productivity in the field, in addition to the much-studied topic of outplanting success. This thesis is part of a larger study that examines the relationships between morphological, biochemical and physiological parameters in lodgepole pine seedlings in relation to outplanting success and subsequent field performance. The focus is on seedling roots.

In this thesis, the following were assessed in seedlings both prior to, and after cold storage: root morphology, root growth capacity (RGC), viability (TTC reduction), reducing, total soluble and total non-structural carbohydrates, starch, and drought tolerance. In addition, the effect of slow and fast cooling as seedlings enter into cold storage was examined.

Seedling survival and subsequent field performance was best predicted by root viability as measured by TTC reduction prior to cold storage. There was a good correlation between TTC reduction and survival, collar diameter and growth increment. In addition, the results demonstrated that the method had general applicability, as the regression equations were very similar between data sets. RGC was also a good predictor; however, the test takes far longer, requires more space and must be done after cold storage. The results also showed that measurements of total soluble carbohydrate and total non-structural carbohydrate after cold storage have potential as predictors of survival and growth.

Drought stress tolerance is needed if seedlings are to survive lifting, transport and outplanting. The results showed that drought stress tolerance, measuring determined as RGC or needle injury after a 10-day drought, correlated with outplanting survival and field performance. Despite the fact that the roots are more sensitive to drought stress and are essential for water uptake during drought, the correlation between root injury, measured as electrolyte leakage, and seedling performance was not good.

The relationship between drought stress tolerance, measured as electrolyte leakage, and carbohydrates was also examined. A strong inverse correlation was found between both total soluble and total non-structural carbohydrates and root injury. As the regression equations were similar over the two year study, it can be concluded that measurements of these carbohydrate pools provides a very good indication of drought stress tolerance in lodgepole pine seedling roots.

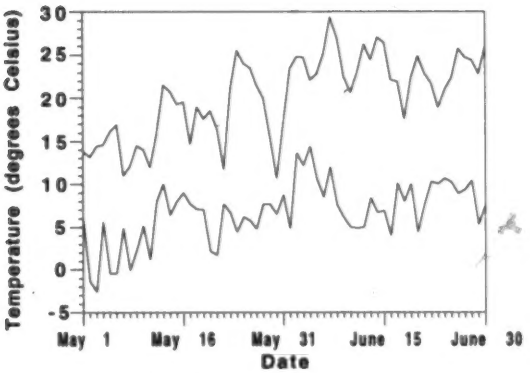
Finally, the effect of cooling rate as seedlings enter into cold storage was examined in a preliminary study. This portion of the study is ongoing, but to date, it appears that increasing the rate of cooling does not improve seedling health as measured by carbohydrate content and RGC.

Based on this research, TTC reduction prior to cold storage appears to be a good indicator of seedling preparedness for cold storage. Total soluble carbohydrate and total non-structural carbohydrate in the root also look promising. Therefore, research on these potential indicators should continue, in order to determine their possible application in industrial purposes.

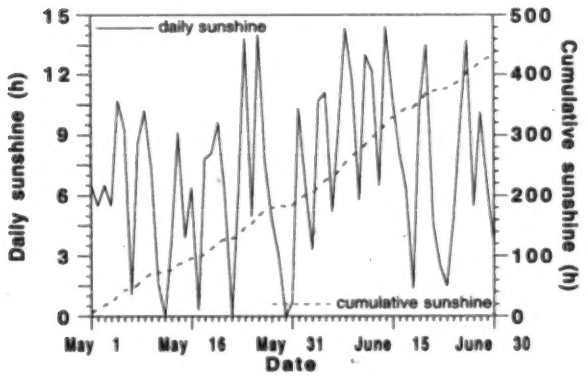
There is still a lack of knowledge on seedling acclimation following outplanting. This is mainly due to the difficulty of studying plants in their natural environment. There is a need for appropriate indicators of stress tolerance. Do root total soluble carbohydrate and total non-structural carbohydrate have this potential?

There are indications that fluctuation in total soluble carbohydrate in roots during cold storage reflect the physiological status of the seedling. Research should be initiated on total soluble carbohydrate content in association with chilling history, dormancy, frost hardiness and osmotic adjustment following cold storage.

APPENDICES

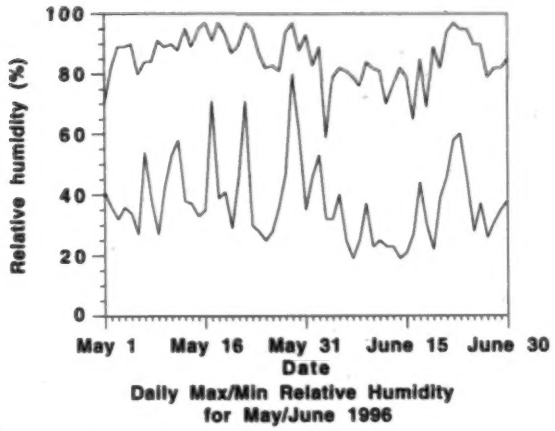


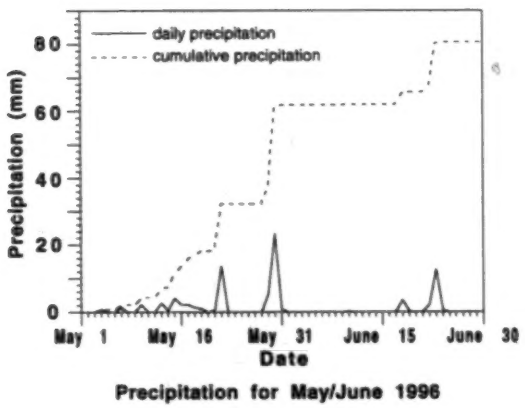
Daily Max/Min Temperatures
for May/June 1996



Sunshine for May/June 1996

Appendix I: Daily Max/Min temperatures and sunshine for May/June 1996





Appendix II: Daily Max/Min relative humidity and precipitation for May/Juni 1996

10 Day Planting Weather Summary

Parameters	Planting date 22 May	5 June	20 June	Notes
Bright sunlight hours	55	104	72	Equivalent to number of hours greater than 600µmol/m²/sec
Days with precipitation	5	2	5	
10-day precipitation (mm)	43	0.2	15	Total percipitation in 10 days following planting
VPD (vapour point depression)				
Low days	1	0	0	Day time maximum VPD<0.2 Kpa
High days	4	0	2	Day time maximum VPD0.5-1 Kpa
Extreme days	5	10	8	Day time maximum VPD>1.0 Kpa
Days with frost	1	0	0	0 - -2.0
Growing temperatures				
Quiescent days	3	0	0	Daily maximum temperature 6-12°
Low days	4	2	4	Daily maximum temperature 12-18°
Moderate days	3	7	6	Daily maximum temperature 18-23°
Optimum days	0	1	0	Daily maximum temperature 23-27°

Appendix III: 10 day planting weather summary

One-way ANOVA table

Source	DF	Mean Square	F Value	Pr>F
Model	5	867.94	36.01	0.0001
Error	54			

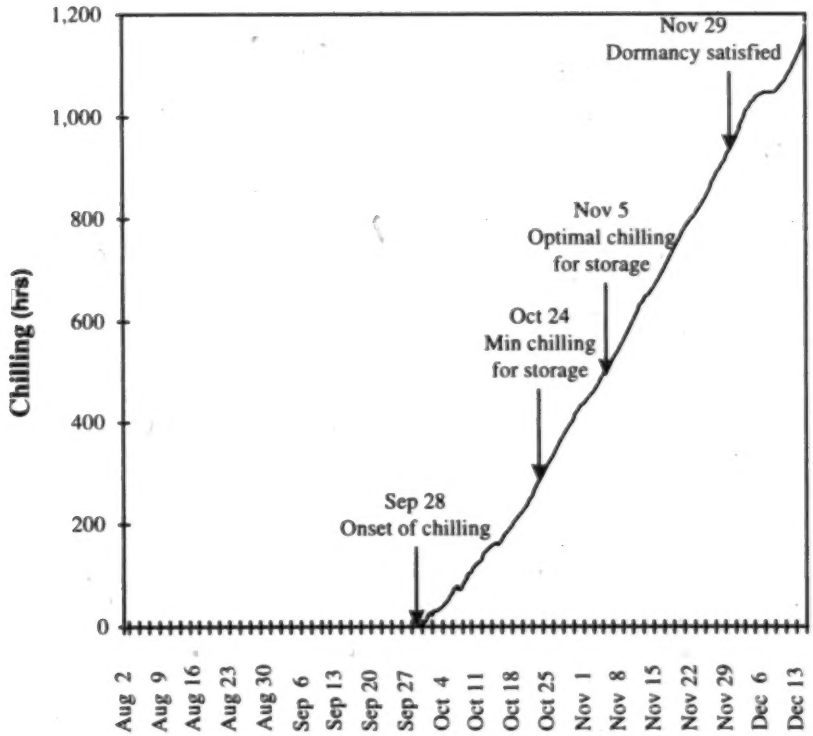
Source	DF	Mean Square	F Value	Pr>F
Date	5	867.94	36.1	0.0001

Two-way ANOVA table

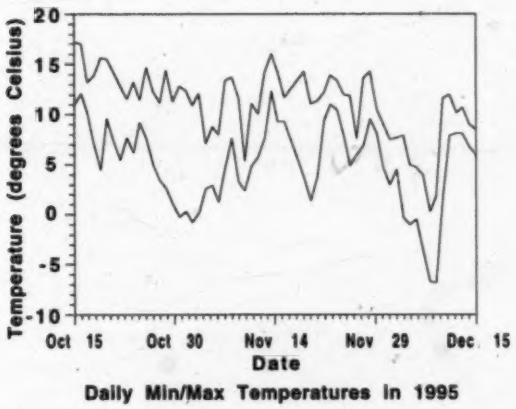
Source	DF	Mean Square	F Value	Pr>F
Model	11	1968.12	26.78	0.0001
Error	108	73.50		

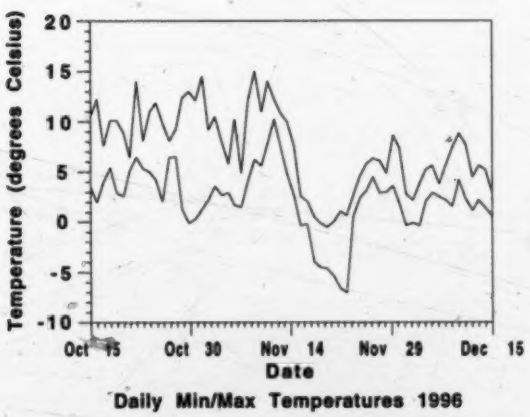
Source	DF	Mean Square	F Value	Pr>F
Treatment	1	6008.23	81.74	0.0001
Lifting date	5	2527.89	34.4	0.0001
Treatment* Lifting date	5	600.33	8.17	0.0001

Appendix IV: One-way ANOVA table for percentage of white roots presented in Figure 3.1b and two-way ANOVA for viability before regrowth presented in Figure 3.3 a.

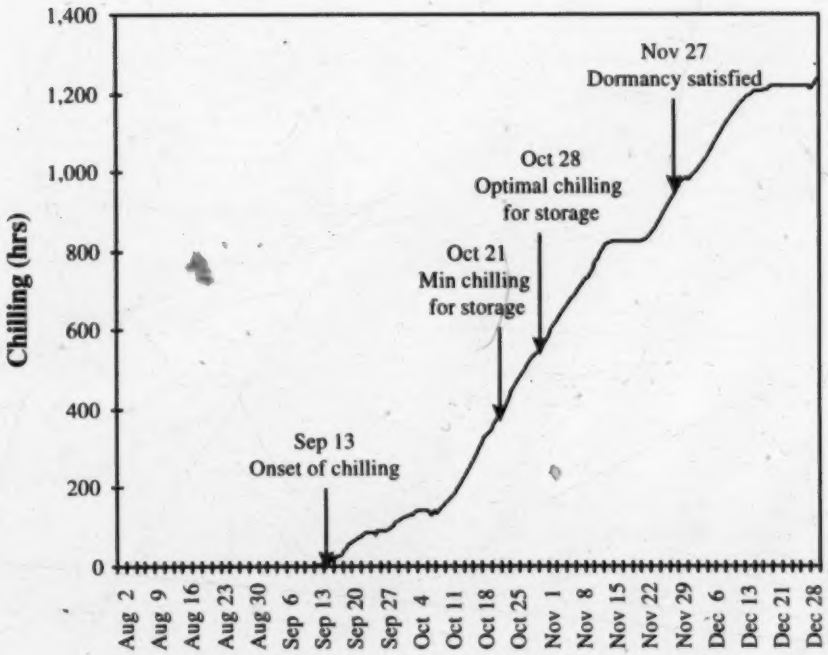


Appendix V: Static model for
chilling hours in 1995





Appendix VI: Daily Max/Min temperatures for October/December 1995 and 1996



Appendix VII: Static model for chilling hours in 1996